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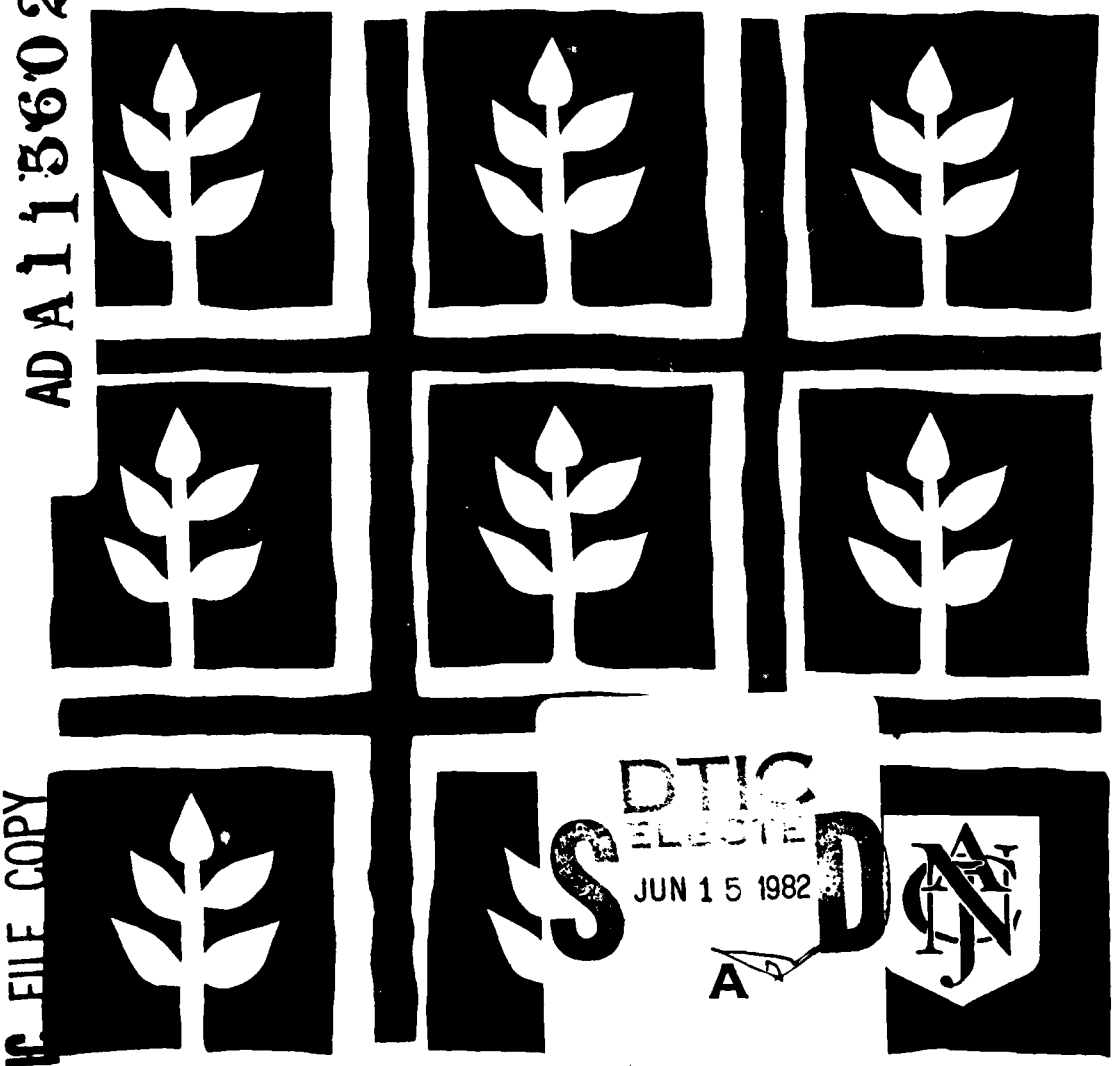

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Single nutrients and immunity[1, 2]

William R. Beisel,[3] *M.D.*

I. Introduction

Soon after their discovery, vitamins were recognized for their importance in host resistance against infectious illness (1). Vitamins and other single nutrients were also shown to influence host immunological functions (2). It subsequently became evident that generalized forms of severe malnutrition, i.e., kwashiorkor and marasmus, were often accompanied by problems of anergy. Such nutritionally induced immunological defects are acquired rather than inherited, are largely functional, and are generally eliminated when the nutritional deficits are corrected. Clinical awareness of this problem has increased, with a primary focus on problems seen during generalized protein and energy deficiencies (3, 4). Although inherited immunodeficiency disorders attract most interest among clinical immunologists, the prevalence of inherited immune defects is low (5). In contrast, acquired immunodeficiencies due to malnutrition are common in impoverished populations throughout the globe (2–4). Functional anergy may also emerge as a secondary consequence of malnutrition during severe or protracted medical and surgical illnesses (6).

Severe protein-energy malnutrition (PEM) does not generally occur without concomitant deficiencies of one or more micronutrients. However, relatively little effort has been expended to determine the role of single nutrients in influencing immune system functions. Few reviews even mention this point (3, 6–11). Because of the potential clinical importance of single nutrient deficits or excesses on immune system functions, a workshop was sponsored by the Food and Nutrition Board of the American Medical Association (12). The workshop served to emphasize the diversity of the subject, the magnitude of unsolved problems, and the complexity of interrelationships requiring consideration. Major gaps currently exist in knowledge about single nutrient deficits or excesses in terms of their effects on specific functional components of the immune system.

Although a brief, clinically oriented report (12) emerged from the workshop, the immunological importance of single nutrients suggested that the subject be evaluated in its broader perspectives. Accordingly, this review considers the strengths and weaknesses of available data concerning single nutrient effects on immunity. No attempt has been made to reassess the influence of generalized PEM on immune system functions.

It has not been easy to design consistently applicable and interpretable experiments. The need to include "pair-fed" control groups in studies of single nutrient deficiencies was recognized early (1, 3, 8) as a necessary approach to problems of inanition in experimental groups fed an inadequate diet. Even the use of pair-fed controls does not insure that observed effects can be attributed with certainty to the variable under study. Infections frequently develop when experimental animals are given restricted diets, but they may go unrecognized or be ignored. Infections can have profound effects on immunological responses, and observed changes in infected animals may be ascribed erroneously to the nutritional variable under study. Further, neither the antigens nor the measurement techniques used to evaluate an immune response have always been sufficiently precise to justify some of the assumptions made in interpreting data emerging from reported studies. Thus, many of the published concepts about nutrient-immunological interaction must be reexamined and subjected to further study.

[1] From the United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701.

[2] The views of the author do not purport to reflect the positions of the Department of the Army or the Department of Defense.

[3] Deputy for Science.

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II. Water soluble vitamins

As early as 1921, Cramer et al. (13) observed that the removal of water-soluble group B vitamins from the diet of rats or mice led to reversible lymphopenia and atrophy of lymphoid tissues. Many individual B group vitamins are now known (12) to have unique effects on the competence of the immune system; these include pyridoxine, pantothenic acid, riboflavin, folate, and vitamin B_{12} (see **Table 1**). Other B group members, i.e., thiamin, biotin, and pteroylglutamic acid, have less pronounced effects.

A. Thiamin

Rats with serious deficiencies of thiamin seldom show immune system impairment (14–16). However, Kumar and Axelrod (17) observed an inhibition in splenic plaque-forming cell formation in thiamin-deficient rats after immunization with sheep red blood cells (RBC).

B. Pteroylglutamic acid

Depression of the hemagglutinating (HA) antibody responses to human RBC or diphtheria toxoid occurred in rats with an isolated deficiency of pteroylglutamic acid (15, 18).

C. Biotin

Biotin deficiencies in rats lead to an impaired HA antibody response to diphtheria toxoid (15), and a reduced development of splenic plaque-forming cells after inoculation with sheep RBC (17). Cowan et al. (19) recently studied siblings with CNS dysfunction, immunodeficiencies, conjunctivitis, and alopecia. These combined disorders were attributed to an inherited deficiency of biotin-responsive carboxylase enzymes required for the catabolism of branched-chain amino acids. The immune system defects included low serum IgA concentrations, lack of antibody production after pneumococcal polysaccharide vaccine, absence of delayed dermal hypersensitivity (DDH) reactions, and a diminished proliferative T-cell response to in vitro stimulation with Candida antigens (19). The immunological derangements shown by children with this enzyme abnormality have several plausible explanations. These include an isolated functional deficiency of biotin, an amino acid imbalance, metabolic pathway blockade because of the defective enzymes in lymphoid cells, the presence of chronic infections, or some combination of these possibilities. This extensive combination of immunodeficiencies has not been reproduced experimentally by an isolated absence of biotin.

D. Pyridoxine

Pyridoxine is required for normal nucleic acid and protein synthesis, and for cellular multiplication. It is therefore not surprising that isolated pyridoxine deficiencies cause more profound effects on immune system functions than deficiencies of any other B group vitamin (12, 20). Unlike the deficiencies of most other B group members, lack of pyridoxine appears consistently to inhibit cell-mediated immune functions as well as humoral responsiveness to a variety of test antigens (20). Experimentally induced pyridoxine deficiency has been created by the use of pyridoxine-deficient diets, or more rapidly by the use of a pyridoxine antagonist, deoxypyridoxine. The latter can be administered alone or in combination with a pyridoxine-deficient diet.

Pyridoxine deficiencies in man produce dermatitis of the face, neck, and extremities, and oral lesions including cheilosis, glossitis, and stomatitis. These lesions are potential sites for secondary bacterial or fungal infections (8, 21). Blood cell changes may include lymphocytopenia and eosinophilia (21).

1. Lymphoid tissue effects. A decrease in thymic weight occurs in vitamin B_6 deficient rats as compared to pair-fed controls (14, 22). Splenic hypoplasia was also observed in fetuses of pregnant rats fed a vitamin B_6-deficient diet or given deoxypyridoxine (23). The pups of pyridoxine-deficient rat dams had diminished numbers of circulating blood lymphocytes, fewer plaque-forming cells in the spleen, and a diminished pyridoxine content of splenic and thymic lymphocytes (24). In contrast, Koros et al. (25) reported an increase in the background numbers of splenic plaque-forming cells in mice on pyridoxine-deficient diets. Severe lymphoid hypoplasia due to B_6 deficiency is reflected by lymphopenia in many species (9).

2. Humoral immunity. Pyridoxine deficiencies are consistently accompanied by impaired serological responses to test antigens. Pyridoxine-deficient rats showed a dimin-

TABLE 1
Immune function changes in B-group vitamin deficiency states

	Pyridoxine	Pantothenic acid	Riboflavin	Biotin	Folic acid	Cobalamine	Thiamin	Pteroylglutamic acid
Lymphoid tissues	Generally atrophic	Generally normal						
Lymphocyte counts	May be depressed				May be depressed	Normal		
Antibody production after immunization	Consistently depressed primary and secondary responses	Generally depressed	Primary response generally depressed	May be depressed	May be depressed			May be depressed
Splenic plaque-forming cell response to immunization	Depressed	May be depressed		May be depressed	May be depressed		May be depressed	
In vitro lymphocyte responses	Diminished in mixed cultures				May be depressed	May be depressed		
	Normal stimulation by LPS							
Delayed dermal hypersensitivity	Depressed, although sensitization mechanism may be intact				May be depressed			
Allograft survival	Prolonged							
Neutrophil functions	Inflammatory response may be diminished				Apparently normal	May be depressed		
Other	No change in anaphylactic responses							

ished HA antibody response to immunization with sheep or human RBC (22, 26, 27), to primary and secondary inoculations with diphtheria toxoid (15, 20), and to influenza virus infection (16). Guinea pigs showed a diminished HA antibody response to diphtheria toxoid (28). Antibodies produced by pyridoxine-deficient animals have a diminished in vitro binding affinity for diphtheria antigens (20).

Many studies of single nutrition deficiency states have included the inoculation of foreign RBC as an antigen that is easy to acquire, quantitate, and administer. It is important to recognize, however, that the immune response to a RBC inoculum is a complex phenomenon. It requires an initial participation of T-helper lymphocytes in order to generate a full B-cell response. Later in the response, an effect of T-suppressor lymphocytes becomes evident as a mechanism for regulating the final production of hemolysins and hemagglutinins (29).

Human volunteers subjected to pyridoxine-deficiencies, showed diminished humoral antibody responses to tetanus toxoid and typhoid vaccine (30). When the pyridoxine-deficiency was combined with a pantothenic acid-deficiency in other volunteers, virtually no detectable antibody response occurred to the same vaccine antigens (31).

A deficiency of pyridoxine did not alter or diminish anaphylactic reactions of guinea pigs when bovine serum albumin was used as both immunizing and challenging antigen (32).

3. Cell-mediated immunity. Using normal and pair-fed control guinea pigs, Axelrod et al. (28, 33) found that pyridoxine deficiency produced by dietary manipulation alone, or by the use of deoxypyridoxine, led to an impairment of dermal responses against antigens to which the test animals had previously been sensitized. Guinea pigs sensitized to diphtheria toxoid showed a diminished early Arthus-type hypersensitivity reaction to intradermal (id) diphtheria antigen (28). Pyridoxine-deficient guinea pigs also showed a diminished DDH response to id purified protein derivative (PPD) despite previous sensitization with tuberculin or infection with BCG vaccine organisms (20, 33). However, the sensitization mechanism was not inhibited in the deficient guinea pigs. If sensitized during the period of pyridoxine deficiency, guinea pigs regained normal DDH responsiveness after the deficiency was corrected. Further, lymphocytes from donors sensitized during a pyridoxine-deficient state were able to transfer tuberculin sensitivity to normally nourished recipients (33). Failure to manifest a DDH reaction to PPD could not be explained by an inability of pyridoxine-deficient guinea pigs to generate an acute inflammatory response, inasmuch as deficient animals showed a normal reaction after id histamine (33). On the other hand, it may not be correct to make direct comparisons between DDH and inflammatory reactions. The populations of cells that respond rapidly to a phlogistic chemical differ from those that respond during the generation of a slowly evolving DDH reaction.

The evidence that pyridoxine deficiency can impair cell-mediated immune responses is strengthened by studies showing a prolongation of allograft survival in animals with isolated pyridoxine deficiencies. Skin allografts in rats survive much longer if the recipient is made deficient in pyridoxine, either by dietary manipulation or through the use of deoxypyridoxine (34). Trakatellis and Axelrod (35) used pyridoxine-deficient mice in a successful attempt to induce tolerance to skin grafts by the prior administration of splenic cells from the skin donor. Immune tolerance achieved by donor spleen cell inoculations was far more effective in prolonging subsequent skin graft survival if the recipient mouse was also deficient (35). Pyridoxine-deficient CBA/J mice inoculated with spleen cells of C3H/HeJ mice were still able to accept C3H/HeJ skin grafts after the deficiency was cured. In contrast, grafts failed to survive if recipient mice were pretreated with autologous BCA/J spleen cells from a C3H/HeJ donor previously sensitized to BCA/J tissue.

Robson and Schwarz (36, 37) studied thoracic duct lymphocytes from rats made pyridoxine deficient by a combination of diet manipulation plus deoxypridoxine. Duct lymphocytes either from deficient female rats or their pups at 3 months of age, showed a diminished ability to respond in vitro in mixed lymphocyte cultures; the thoracic duct lymphocytes also showed a diminished ability to incorporate [^{3}H]uridine under basal in vi-

tro conditions or to transfer sensitivity to normal recipient rats.

In contrast to the DDH and graft rejection studies which suggested abnormalities in cell-mediated immunity during pyridoxine deficiency, differences were not found in the in vitro reactivity of splenic cell lymphocytes to lipopolysaccharide (LPS) stimulation (38). Other mitogens or specific antigens have not been used to test spleen cells of pyridoxine-deficient animals and no attempts have yet been made to investigate the functional competence of various T-lymphocyte subpopulations.

In a single study in man (39), a diminished reactivity of lymphocytes in mixed cultures was ascribed to pyridoxine deficiency in uremic patients; lymphocyte abnormalities were reversed by oral administration of this vitamin.

E. Pantothenic acid

Pantothenic acid deficiencies depress humoral antibody responsiveness to various antigens in experimental animals or man, but have not been shown to impact adversely on cell-mediated immunity.

1. Lymphoid tissue effects. Stoerk and Zucher (14) first observed a decrease in thymic weight if rats were fed a pantothenic acid-deficient diet. Pantothenic acid-deficient chickens showed a reduced HA antibody response after the administration of *Salmonella pullorum* antigens, although no losses in thymic, spleen, or bursa weights were evident (40).

2. Humoral immunity. Impaired HA antibody responses in deficient rats immunized with human RBC (26, 41) were restored by the administration of either panthenol or pantothenic acid. Deficient rats also showed a markedly diminished hemagglutination-inhibition (HAI) response to influenza virus infections, whereas pair-fed control rats experienced an entirely normal immunological response to the same challenge (16).

Rats deficient in pantothenic acid may show increased basal numbers of splenic plaque-forming cells (25), but the number of these cell does not increase after immunization with sheep RBC (42). A concomitant deficiency in hemolysin antibody formation was not corrected by a 10-fold increase in the immunizing dose of sheep RBC but was prevented entirely by correcting the pantothenic acid deficit. Reticuloendothelial system (RES) functions were not impaired in the pantothenic acid-deficient rats (42), but serum properdin values were markedly reduced (8).

In 1962, Hodges et al. (30, 31, 43) gave two volunteers a pantothenic acid-deficient diet and two others a pantothenic acid antagonist, i.e., ω-methyl-pantothenic acid; all responded normally to administration of typhoid vaccine. Their antibody responses to influenza vaccines and tetanus toxoid were slightly diminished. These volunteers also showed a normal rapid rejection of heterologous skin grafts. However, five other volunteers studied during combined deficiencies of pantothenic acid and pyridoxine developed a declining concentration of IgG in serum and showed markedly diminished humoral responses to tetanus and typhoid vaccines; in contrast, they had an excellent response to a live oral poliomyelitis vaccine.

F. Riboflavin

On an immunological basis, riboflavin deficiency in animals resembles that of pantothenic acid, with a diminished ability to generate humoral antibodies in response to test antigens. It is not known if riboflavin deficiency impairs cell-mediated immune responses.

Rats fed a riboflavin-deficient diet showed decreases in thymic weight (14), and depressed HA antibody responses to human RBC (26) or diphtheria toxoid (15). The primary complement-fixing antibody response of riboflavin-deficient rats was impaired after a single dose of *Rickettsia typhi* antigen. However, with two additional doses of the same antigen, titers reached values equivalent to those of both control and pair-fed groups (44). Chicks fed a riboflavin-deficient diet also showed diminished HA titers 7 to 8 wk after inoculation with *S. pullorum* antigens (41), but no significant changes were detectable in thymic, splenic, or bursal weights.

G. Folic acid

Deficiencies of folic acid lead to a reduction of host resistance and to impaired lymphocytic functions in both man and experimental animals.

Infant guinea pigs fed a folic acid-deficient

diet for 2 wk showed a 89% mortality when inoculated with 10^9 *Shigella flexneri*, a dose causing no deaths in control groups (45). If folic acid was removed from the diet for 2 wk and then replaced, guinea pigs resisted challenge with *S. flexneri* in a normal manner (46).

1. Humoral immunity. The production of complement-fixing antibodies was reduced in rats with severe folic acid deficiency after inoculation with formalinized *R. typhi* in comparison to responses in normal or pair-fed rats (44). This immunological deficiency could be overcome either by increasing the dose of antigen or by administering it three times.

Williams et al. (47) fed Wistar-Lewis rats a folate-deficient diet subsequent to weaning. These rats developed mild megaloblastic changes in the bone marrow, low serum folate concentrations, a smaller thymic size, and a reduction in the number of T-cells in the spleen and peripheral blood. Splenic lymphocytes showed a diminished mitogenic response after exposure to phytohemagglutinin (PHA) in vitro and poor cytotoxic activity when cultivated in vitro in the presence of heterologous thymocytes (47). The number of plaque-forming cells in the spleen of folate-deficient rats was reduced after sheep RBC inoculation when compared to responses of control or pair-fed rats (9, 17).

2. Cell-mediated immunity. A course of methotrexate, a folic acid antagonist, will block the development of contact sensitivity reactions in mice (48). After 8 wk on a folate-deficient diet, rats showed a reduced dermal sensitivity response to PHA, significant decreases in T-cell populations in the spleen and peripheral blood, and impaired in vitro lymphocyte responses to mitogenic stimulation and cytotoxicity testing (9).

Patients with megaloblastic anemia due to folic acid deficiency exhibit several changes in immune function (49). DDH responses to dinitrochlorobenzene (DNCB) are impaired as is the ability of PHA to stimulate proliferative changes in peripheral lymphocytes. These responses can be normalized by appropriate doses of folic acid.

Lymphocytes of patients with folic acid deficiency did not show a normal in vitro suppression of PHA-stimulated [^{3}H]thymidine incorporation into DNA in the presence of added deoxyuridine (50). Appropriate in vivo treatment corrected this defect in approximately 3 months. However, if folic acid was added to lymphocyte cultures in vitro, an immediate correction of the suppressive effects of deoxyuridine was obtained. This implied that unstimulated circulating lymphocytes did not take up folate in vivo. Methotrexate, a folate antagonist, has been shown to diminish the intracellular pools of thymidine triphosphate and deoxyadenosine triphosphate in human lymphocytes and thereby to interfere with DNA synthesis (51).

3. Phagocytic cells. Patients with a megaloblastic marrow due to folic acid deficiency showed normal phagocytic and bactericidal activities of neutrophilic leukocytes as well as a normal ability of the phagocytic process in these cells to activate the hexose-monophosphate (HMP) shunt (52). Nuclei, however, often show hypersegmentation.

H. Vitamin B_{12}-(cobalamin)

Because of its essential molecular role, vitamin B_{12} must be assumed to contribute to the support of immune system functions and the adequacy of other host defensive mechanisms. Since vitamin B_{12}-deficiency can not be induced in experimental animals, attempts to discover its role on immune functions must be performed in patients with untreated primary pernicious anemia (PPA).

Untreated PPA is relatively uncommon and few immunological tests have been performed. An additional problem in the interpretation of available data is created by the need to differentiate a deficiency of B_{12} from one caused by coexisting cellular deficiencies of folate, inasmuch as a folate-requiring pathway for the transformation of formate to serine in white blood cells is dependent upon the presence of vitamin B_{12} (53).

Kaplan and Basford (52) studied patients with untreated vitamin B_{12} deficiency and a megaloblastic marrow whose peripheral blood neutrophils showed a diminished in vitro ability to phagocytize or kill *Staphylococcus aureus* or to initiate HMP shunt activation during phagocytic activity; these functional defects were reversed when the patients were treated with vitamin B_{12}.

1. Lymphocyte studies. Studies of lymphocyte function in untreated PPA patients are scanty. However, MacCuish et al. (54) compared the lymphocytes of 20 patients with PPA to those of 20 controls. Although pe-

ripheral T-lymphocyte percentages did not vary significantly, there was a diminished uptake of [^{3}H]thymidine by peripheral lymphocytes cultured in vitro when stimulated with the mitogen PHA. Tai and McGuigan (55) studied the ability of antigens, including gastric juice, gastric mucosal homogenates, and intrinsic factor to produce an in vitro transformation of peripheral blood lymphocytes from 29 patients with untreated PPA. No functional deficiencies were found. Das and Herbert (50) observed that lymphocytes from patients with untreated PPA did not show the expected, i.e., normal, suppression of thymidine incorporation into DNA when stimulated by PHA in the presence of deoxyuridine. The direct in vitro addition of B_{12} to cultured lymphocytes promptly corrected this defect. In contrast, lymphocytes obtained from B_{12}-treated patients did not attain a normal response for approximately 3 months unless B_{12} was added in vitro.

I. Comments on vitamin B group studies

Although most basic studies of individual vitamin B deficiencies were done more than a decade ago, pair-fed controls were used and experimental designs were sufficiently sound to certify the occurrence of immune system abnormalities. The incidence of isolated vitamin B deficiency states is extremely low in modern societies, but combined multinutrient deficiencies undoubtedly include some B group members. Future research to identify the molecular basis for individual B vitamin effects on immune system functions will need to be done primarily in experimental animals or cultured cells. It may be possible to exploit differences among individual B vitamins in terms of those which have predominant effects on nucleic acid synthesis versus those with other metabolic pathway targets. Other comparisons may be directed toward combined versus isolated deficiency effects, or toward possible effects on phagocytic cells. This focus of work is of potential importance for helping to disentangle and elucidate the intricacies which characterize the immune process.

J. Vitamin C (ascorbic acid)

Of all essential micronutrients, vitamin C has undoubtedly generated the greatest recent interest concerning its interactions with host defensive mechanisms and the immune system (see **Table 2**). New investigations in both man and experimental animals have followed the widely popularized claims of Pauling (56) that adult humans should maintain an ascorbic acid intake of 1.0 g or more each day. This theory has stimulated continuing debates concerning the normal size of ascorbic acid pools within the human body and the daily rate of ascorbic acid turnover (57). The most extensive data deal with the content of ascorbic acid in white blood cells (see **Table 3**). The content of ascorbic acid in peritoneal macrophages and mast cells is also quite high (58). However, the concentration of leukocyte ascorbic acid alone is not a reliable guide for estimating the tissue status of this vitamin in normal individuals (59).

1. Megadose administration. To determine if the claims of Pauling (56) were valid, clinical trials were conducted in volunteer subjects using a variety of experimental designs and internal controls. Fourteen such studies dealing with the ability of prophylactic megadoses of vitamin C to prevent common upper respiratory illnesses were reviewed by Chalmers in 1975 (60) and by Thomas and Holt in 1978 (61). Data from eight studies considered creditable showed only minor and insignificant effects from the use of large doses of ascorbic acid to prevent or treat the common cold, although in most studies the severity of symptoms was reduced (60).

In a recent additional report (62), 362 volunteers were studied for 72 days with 97% completing the study. A group given a daily excess of 80 mg of vitamin C showed 14 to 21% fewer respiratory symptoms and had somewhat more "episode-free" subjects than a control group receiving a placebo. However, this study was interpreted by its authors as one failing to support the need for the administration of prophylactic megadose ascorbic acid supplements to well nourished adults, since the 80-mg dose achieved the same types of success claimed in megadose studies. Based on reviews (60, 61) and other editorial comments (63, 64), the present consensus of scientific opinion fails to support the view that ascorbic acid supplementation using 1 to 2 g/day doses is effective in reducing susceptibility to common colds. At best, such doses may lessen the severity and/or duration of symptoms.

Prinz et al. (65) reported a slight but statistically significant increase in serum IgA, IgM,

TABLE 2
Vitamin C interactions with immune functions

	Vitamin C excess	Vitamin C deficiency
Host susceptibility to infection	Duration of symptoms in common colds may be reduced	Increased
Lymphoid tissues		Unchanged
Lymphocyte counts	B-cell percentages may decrease	T-cell percentages may decrease
In vitro lymphocyte transformation	May be enhanced by PHA and Con A	
Serum immunoglobulin concentration	Unchanged or increased	
Antibody production	Unchanged	Unchanged
Allograft survival		Prolonged
Delayed dermal hypersensitivity		Recall mechanism suppressed but normal sensitization remains
Experimental allergic encephalitis		May be suppressed
Neutrophil:		
a. Chemotaxis	Some congenital deficiencies may be improved; Normal cells may be stimulated	Mobilization and in vitro motility are impaired
b. Bactericidal activity	Controversial: Enhancement and suppression have each been reported	Normal
c. Metabolic activity	Generally increased	May be reduced
d. cGMP content	May be increased	
Macrophages	Motility may be stimulated	Mobilization is impaired. Size and motility are reduced. Fragility is increased
Complement concentrations	Some studies report increases	
Interferon production		May be enhanced
Thymic humoral factors		May be deficient
Anaphylactic response		Normal

TABLE 3
Factors that alter neutrophilic ascorbic acid content

A. Reductions seen in:
- Infections: (best documented in respiratory virus infections.)
- Leukemias: acute
  chronic myeloid or lymphoid
- Lymphomas
- Hematologic disorders: myelofibrosis
  polycythemia
  leukocytosis
  purpura
- Drug therapy: corticosteroids
  cytotoxic drugs
  other immunosuppressive drugs
  contraceptive drugs
- Pregnancy
- Maleness
- Aging
- Scurvy
- Phagocytic activity (in vitro)

B. Increases seen with:
- Ascorbic acid supplementation
  - in vivo: maximal concentrations achieved with 100 mg/day doses. Reverses corticosteroid-induced depression.
  - in vitro: uptake achieved by media supplementation.

and C3 values of 25 volunteers fed 1 g/day ascorbic acid for 75 days.

In comparison with a control group, cotton-topped marmosets fed a large dietary excess of vitamin C for 108 days before virus inoculation with parainfluenza III virus of marmoset origin showed an identical incidence of infection and length of incubation period (66). The vitamin C-treated marmosets had less clinical illness; mortality was 36% in comparison to a mortality rate of 57% in controls. The probability that this difference in mortality could have occurred by chance alone is 23%.

Thomas and Holt (61) also reviewed the role of ascorbic acid in influencing immune responses to standardized antigens, complement system function, and the cellular production of interferon. They commented (61) that "the literature in this field is bedeviled by controversy and lack of confirmation." Studies performed a decade ago were equally controversial with respect to the influence of ascorbic acid on the bactericidal mechanisms of neutrophils (67, 68). On the one hand, ascorbic acid was said to enhance bactericidal activity through a synergistic effect with H_2O_2 and lysozyme (67), but conversely, 0.01 *M* concentrations of ascorbic acid inhibited H_2O_2-myeloperoxidase-halide interactions (68). Still more recently, megadose administration of vitamin C to normal subjects was said by one group (69) to stimulate the HMP activity of resting leukocytes, but to significantly impair bactericidal activity, while others (70) found the postphagocytic HMP shunt activity and myeloperoxidase-mediated iodination of ingested protein to remain unaltered.

2. Lymphoid tissue effects of deficiency. There is little evidence that vitamin C plays a special role in the function of lymphoid series cells. Although widespread atrophy of lymphoid tissues is a common consequence of generalized protein-energy malnutrition or deficiencies of many individual micronutrients, lymphoid atrophy has never been described in scorbutic animals or man. Further, severe scurvy does not alter lymphocyte counts in peripheral blood (71). On the other hand, Anderson et al. (70) have recently detected a stimulation of lymphocyte transformation by PHA and ConA after the daily ingestion of 1 to 3 g of ascorbate.

3. Humoral responses. When tested with a variety of antigens in animals with severe vitamin C deficiencies, humoral immune responses do not differ appreciably from those of control or pair-fed groups. Perla and Marmorston (1) observed that severe scurvy did not prevent the formation of antibodies although the effectiveness of diphtheria and tetanus toxoids was somewhat diminished. Kumar and Axelrod (72) found normal primary and secondary responses to diphtheria toxoid in scorbutic guinea pigs. Normal antibody responses also followed the immunization of scorbutic guinea pigs or mice with heterologous RBC (73, 74). Guinea pigs with subclinical deficiencies of vitamin C exhibit normal agglutinin responses after administration of attenuated S19 *Brucella abortus* vaccine (75).

The long-term feeding of cotton-topped marmosets with a dietary excess of vitamin C did not alter their humoral immune response to an intranasal inoculation with parainfluenza 3 virus (66). Similarly, the addition of excess vitamin C to the drinking water of BALB/c mice had no effect on splenic plaque-forming cell development after the administration of sheep RBC (74).

Serum concentrations of IgG, IgA, and IgM were not altered by megadose administration of ascorbate to volunteers (70).

4. Cell-mediated immunity. The influence of ascorbic acid in cell-mediated immune functions has not been entirely clarified. Perla and Marmorston (1) noted decreased DDH responses to diphtheria toxin or tuberculin in severe scurvy. Mueller et al. (76) reported that scorbutic guinea pigs failed to develop allergic encephalitis after immunization with brain tissue antigens mixed with a mycobacterial adjuvant. Protection related directly to the length of vitamin C deficiency but inversely to the dose of antigen. These scorbutic guinea pigs failed to show a DDH response to tuberculin, although tuberculin responsiveness developed after the vitamin C deficit was corrected (76). This observation suggested that the scorbutic guinea pigs were sensitized normally by the initial injection of mycobacteria, and that the recall mechanism could not be tested effectively because the scorbutic guinea pigs were unable to generate an inflammatory response. In contrast to the recovery of DDH on refeeding, the guinea pigs

did not develop allergic encephalitis after their vitamin C deficiency was corrected. Zweiman et al. (77) also found that scorbutic guinea pigs would not manifest DDH to PPD after sensitization with complete Freund's adjuvant, although their lymphocytes were able to respond to tuberculin in vitro with typical mitosis (78) and to transfer PPD hypersensitivity to healthy control animals. Conversely, however, sensitized lymphocytes from healthy controls did not induce DDH if transferred to scorbutic guinea pigs.

Trakatellis et al. (32) used living BCG mixed in bovine serum albumin to immunize scorbutic guinea pigs. These animals did not show DDH to PPD but they did develop a severe, generally fatal, systemic response if given 5.0 mg PPD intraperitoneally (ip) or a lethal anaphylactic response if given intravenous (iv) bovine serum albumin. Kumar and Axelrod (72) showed that scorbic guinea pigs developed high HA antibody responses to diphtheria toxoid but were unable to produce a normal Arthus-type dermal hypersensitivity response to this antigen. The scorbutic guinea pigs also failed to show an inflammatory response to id histamine. The diminished hypersensitivity response of scorbutic guinea pigs was attributed to an impaired ability to develop an inflammatory response. Severely scorbutic guinea pigs do not develop experimental allergic encephalomyelitis or autoimmune thyroiditis (76); the survival of skin allografts is also prolonged (79). Unfortunately, no studies have addressed the possible role of vitamin C in influencing mast cell activities or the local release of histamine, one of the key mediators or modulators of inflammation.

DDH responsiveness and inhibition of allograft rejection serve as classical indicators of cell-mediated immunity. However, these apparent deficits in scorbutic guinea pigs cannot necessarily be ascribed to abnormalities of immune function. Rather, they have been explained by deficiencies in the mobilization and function of phagocytic cells, and/or by vascular endothelial changes known to be associated with vitamin C deficiency. No attempts have been made in scorbutic guinea pigs to employ DNCB, a chemical with both sensitizing and irritant properties, to help confirm this concept.

Anthony et al. (73) showed that splenic lymphoid cells from severely scorbutic guinea pigs were defective in cytotoxic activities, even though T-rosetting cell numbers were normal.

Despite the uncertainty about the role of vitamin C deficiency in humoral or cell-mediated immunity, recent studies do indicate the existence of some positive effects caused by an excess of the vitamin. Although scorbutic guinea pigs showed an increased percentage of B-cells in their peripheral blood with a concomitantly diminished T-cell percentage, pigs given an excess (250 mg/day) of sodium ascorbate showed the opposite changes (80). In comparison to controls, cultured lymphocytes from guinea pigs given the high dose showed the greatest basal uptake values of [^{3}H]thymidine and the highest stimulated values in the presence of concanavalin A (Con A) or PHA; LPS, however, produced no increase over control lymphocyte responses. The feeding of excess vitamin C to BALB/c mice increased in vitro splenic T-cell responses to the mitogen Con A (74). Human peripheal blood mononuclear cells showed an increased in vitro response to PHA in the presence of added vitamin C, although the in vitro addition of vitamin C produced no increase in basal [^{3}H]thymidine uptake values (81); these effects of vitamin C were able to counteract the depressive effects of influenza A virus on the human mononuclear leukocytes.

5. Phagocytic cell functions. Considerable evidence is available to document an interaction between vitamin C and phagocytic cells. When collected from blood, peritoneal, or alveolar fluids, these cells normally contain high (1 to 2 μg/mg protein) concentrations of vitamin C (61) and under in vitro conditions, leukocytes can take up vitamin C.

Neutrophils have been studied in vitro under conditions of vitamin C deprivation and excess (82–89). Neutrophils from scorbutic guinea pigs produce H_2O_2 and kill staphylococci as well as control cells; ascorbate and dehydroascorbate are both utilized during the phagocytic process (83). However, neither glycolytic activity nor HMP shunt activity increased maximally in neutrophils from scorbutic guinea pigs and the stimulation of NADPH-oxidase activity was depressed (84, 85). The addition of ascorbate to cultures of normal macrophages increases the cellular

concentrations of cyclic GMP (cGMP) as well as HMP shunt activity (86, 87). Although the in vitro addition of ascorbate in very large amounts (0.1 *M*) may inhibit H_2O_2-myeloperoxidase-halide activity, it does not alter the bactericidal capacity of the cells (68). Thomas and Holt (61) found that an increase in the in vitro concentration of ascorbate from 0.1 to 1.0 m*M* increased the phagocytic activity of cultured mouse peritoneal macrophages. The addition of vitamin C to cultures of neutrophils or macrophages also increased their motility and chemotactic activity (70, 87, 88); a dose-related increase in cGMP was noted in the macrophages (87). Greendike et al. (89) reported that the in vitro addition of vitamin C in suitable concentrations increased the erythrophagocytic activity of peripheral blood leukocytes.

The suppression of nitroblue tetrazolium (NBT) reduction in the neutrophils of patients receiving corticosteroid therapy was reversed by oral doses of ascorbic acid (90); this caused NBT scores to normalize in the presence of latex-induced phagocytosis, but without increasing the engulfment of the latex particles.

Boxer et al. (91) showed that the diminished chemotactic and bactericidal activity of neutrophils from an infant with congenital Chediak-Higashi syndrome could be reversed by the prolonged feeding of 200 mg/day vitamin C. Since the cells had a markedly increased content of cyclic AMP (cAMP) prior to vitamin C therapy and since cAMP was known to suppress neutrophil degranulation and motility, the infant was treated with ascorbic acid, a substance found to reduce cAMP concentrations. Boxer et al. (91) postulated further that the inherited cellular defect may have been due to an abnormal assembly of cellular microtubules. In contrast, Gallin et al. (92) were unable to improve neutrophil function in two adult brothers with Chediak-Higashi syndrome by feeding 6 g/day ascorbic acid; however, mice with this syndrome responded to therapy with improvements in leukocytic chemotaxis and bactericidal activity.

Hayward et al. (93) reported a different familian defect in which a failure of the umbilicus to separate in newborn infants led to severe local and disseminated bacterial infections. Two surviving infants had decreased neutrophil mobility; this was improved by the oral administration of ascorbic acid in doses of 200 to 800 mg/day, as well as by the in vitro addition of vitamin C to the defective neutrophils. These authors also attributed the neutrophilic defect to an abnormality in contractile element functions (93).

The ability of human neutrophils to kill *Candida albicans* occurs best at physiological in vitro concentrations of L-ascorbic acid (82). The locomoter functions (i.e., random motion and chemotactic migration) of neutrophils and macrophages are impaired in the absence of vitamin C. Peritoneal macrophages from scorbutic guinea pigs are smaller than normal and show diminished spontaneous activity and slowed rates of migration on glass (88). Goetzl et al. (94) also found that normal tissue concentrations of ascorbate permitted the greatest random activity of neutrophils and eosinophils, as well as the greatest migration after stimulation by kallikrein or complement C5a. Sandler et al. (87) demonstrated that in vitro supplementation with ascorbic acid increased the migration of leukocytes after stimulation with bacterial endotoxin.

Macrophages of scorbutic guinea pigs failed to aggregate in areas of experimental pulmonary silicosis (95) or within the peritoneal cavity; mobilized cells have a diminished content of vitamin C and are abnormally fragile. Ganguly et al. (88) were unable to detect any deficiency in phagocytic ability of peritoneal macrophages obtained from scorbutic guinea pigs, even though the macrophages appeared small and showed poor migratory activity. Pletsityi and Fomina (96), in contrast, noted a transient decrease in the phagocytic activity of neutrophils obtained from rabbits that had been injected iv for 21 consecutive days with 4 ml of a 5% ascorbic acid solution. Phagocytic activity reached a nadir on the 7th day of injections, but reverted to normal as the injections were continued (96).

6. *Other effects.* Although most early studies failed to show a relationship between vitamin C concentrations in blood and complement titers in patients with scurvy, complement titers have recently been shown to increase in rough parallel with doses of excess vitamin C given to experimental animals (61). In contrast, megadoses of ascorbate failed to

initiate changes in serum C'3, C'4, or total hemolytic complement values in normal volunteers (70).

Recent information also suggests that vitamin C may influence the ability of certain cell lines to produce interferon when appropriately stimulated. The addition of 10^{-4} *M* ascorbic acid to mouse L-cell or fibroblast cultures led to a 3-fold increase in the production of interferon stimulated by polyinosinic-polycytidylic acid [Poly(I)·Poly(C)] (97). The addition of ascorbic acid to cultures of human embryo skin or lung fibroblasts led to increased induction of interferon when stimulated by Poly(I)·Poly(C) or by Newcastle disease virus (98). In contrast, vitamin C did not stimulate an increase when Sendai virus was used as the interferon-inducer. Ascorbic acid, administered in vivo to mice, causes a marked increase in the subsequent appearance and circulation of interferon after induction with Poly(I)·Poly(C) (99) or murine leukemia virus (100).

Vitamin C effects have also been shown on other phenomena. Based on flow-volume curves of expired air in 17 healthy volunteers, Zuskin et al. (101) reported that 500 mg of oral vitamin C partially blocked bronchoconstriction caused by injected histamine. Similarly, the in vitro addition of vitamin C to strips of guinea pig trachea reduced the strength of contractions induced by histamine (101). Pletsityi and Fomina (96) administered large amounts of vitamin C iv to rabbits for a 21-day period; a transient depression was noted at 7 days in serum bactericidal activity, properdin concentrations and lysozyme values, but all changes reverted to normal by day 21 of vitamin C administration.

Vitamin C appears to be required by the thymus for the maintenance of certain cells, possibly reticular cells, that are concerned with the production of thymic humoral factor. Dieter (102) fed guinea pigs for 6 weeks diets containing 0.25, 0.5, 1.0, or 5.0 mg of vitamin C. Thymic content of dehydroascorbate decreased in direct proportion to the dietary content of vitamin C. As determined by bioassay, the hormone activity of thymic extracts correlated with thymic ascorbate and inversely with dehydroascorbate.

7. *Comments.* As an essential micronutrient in man, it would be somewhat surprising if vitamin C did not show some effects on immune system functions. Even though most published animal data were generated in an earlier era, they failed to suggest more than a minimal interaction between vitamin C and lymphocytic cell functions. While new studies in animals and cultured lymphocyte populations could be designed with attention to current concepts and technologies, detectable effects are likely to be subtle at best, as judged by available data (70).

On the other hand, a growing body of evidence suggests that vitamin C does play an important role in phagocytic cell functions. The high cellular concentrations of vitamin C and its disease-related fluctuations indicate a potential for cause and effect relationships. Extensive in vitro demonstrations that vitamin C has its greatest effects on phagocytic cell mobility support this concept. Perhaps the clinical evidence that the symptoms and duration of common respiratory infections may be reduced by prophylactic ascorbic acid administration may be related more to a role of the vitamin in influencing phagocytic cell interactions with secondary bacterial invaders than to any direct antiviral effects of vitamin C on the causal respiratory viruses. The most exciting new directions for basic studies at the molecular level are based on the suggestion that ascorbic acid may influence phagocyte mobility by direct effects on the synthesis and assembly of the microtubular structures of these cells (91, 93).

As concluded by Gross and Newberne (9), "although physiologic amounts of ascorbic acid are required for maintenance of metabolic and functional capabilities of phagocytes, too little or too much can adversely affect their overall function."

III. Fat-soluble vitamins

Two of the fat-soluble vitamins, i.e., vitamins A and E, have recognized effects on immune system function. In contrast, there is no evidence that vitamin K plays a role and little evidence concerning vitamin D. Vitamin D deficiency in rats did not alter HA antibody responses to immunization with human RBC (18), but diphtheria toxoid casued a smaller increase in HA titers than that seen in controls (15). Rachitic baby pigs showed a reduced antibody response to *S. pullorum* antigen, but since inanition controls were not

studied concurrently, vitamin D deficiency may not be the valid cause (103).

A. Vitamin A

Cramer et al. (13) did not observe lymphoid system atrophy or lymphopenia in vitamin A-deficient rats or mice. In contrast, chickens deficient in vitamin A show consistently decreased thymic weights (41, 104) with depletion of lymphocytes and plasma cells from the nasal, paranasal, and bursal lymphoendothelial tissues (104, 105). Nauss et al. (106) reported a diminished number of circulating lymphocytes in vitamin A-deficient rats.

1. Host resistance. The tendency of vitamin A deficiency to increase host susceptibility to infection was reviewed by Olson (107) in 1972, and by Darip et al. (108) in 1979 (see **Table 4**). Increased susceptibility is due in part to the role of vitamin A in maintaining the functional integrity of epithelial and mucosal surfaces and the production of mucous secretions. Vitamin A is required for the production of lacrimal, salivary, and sweat gland lysozymes (8). The activity of lysozyme is reduced in leukocytes of vitamin A-deficient children (109).

The influence of vitamin A undoubtedly extends to the immune functions. In a unique experimental design, Darip et al. (108) force-fed rats adequate quantities of protein and calories, while allowing them to become depleted of vitamin A. These rats showed diminished resistance to an intestinal infection with *Angiostrongylus cantonensis*. More larvae penetrated the intestinal mucosa of vitamin A-deficient rats; the rats experienced a greater severity of illness and, in comparison to controls, gained less immunity against subsequent reinfections. Bang et al. (104, 105) observed that vitamin A-deficient chickens were more susceptible than controls to Newcastle disease virus given by intranasal instillation. During the infection, deficient chickens showed a progressive further loss of lymphocytes from the bursal and thymic areas, with granulocyte replacement in these tissues. The nasal mucosa showed a diminished inflammatory response with localized sloughing and increased keratotic changes. Lymphoid depletion also occurred in the marrow and paranasal glands during Newcastle disease virus infection in vitamin A-deficient chicks.

Vitamin-A deficient rats with coexisting PEM were more susceptible to *Plasmodium berghei* malaria than controls (110). The deficient rats exhibited a more rapid occurrence of parasitemia and death, with parasitemia involving 60 to 95% of the RBC. In contrast, pair-fed rats not deficient in vitamin A fared much better, and ad libitum fed controls resisted the infection entirely. Treatment with oral vitamin A allowed the deficient rats to recover.

In addition to an increased susceptibility to infectious diseases, Newberne and Suphakarn (111) observed an increased incidence of colon and liver cancer in vitamin A-deficient rats exposed to dimethylhydrazine or to aflatoxin B.

TABLE 4
Vitamin A interactions with immune functions

	Vitamin A excess	Vitamin A deficiency
Host susceptibility to infection	May be diminished	Increased
Lymphoid tissues		Often atrophic
Lymphocyte counts		May be decreased
Antibody production after immunization	May be enhanced	May be suppressed
Splenic plaque-forming cell response to immunization	May be enhanced	May be suppressed
In vitro lymphocyte transformation	Response to antigens may increase	Response to mitogens may decrease
Delayed dermal hypersensitivity	May be suppressed	May be suppressed
Allograft survival	May be shortened	
RES clearance capacity	Unchanged	
Peritoneal macrophages		Mobilization may be reduced
Hemolytic complement		Concentration in serum may increase
Other	Serves as an adjuvant if injected with antigen	

In contrast to studies of deficient animals, mice inoculated daily with 3000 IU of vitamin A showed an increase in their resistance to infections with *Pseudomonas aeruginosa*, *C. albicans*, or *Listeria monocytogenes* (13, 112).

2. Humoral immunity. Diminished HA antibody responses were noted in vitamin A-deficient rats after administration of diphtheria toxoid or human RBC (15, 18) and in vitamin A-deficient chicks (41) after the administration of *S. pullorum* antigen. Krishnan et al. (113) reported poor HA and hemolysin titer development in vitamin A-deficient rats after a sheep RBC inoculation along with impaired production of splenic plaque-forming cells. These rats also had impaired antibody responses to diphtheria and tetanus toxoids.

In a study performed in Bangladesh, 44 children were given an injection of 200,000 IU of vitamin A in a water-miscible dose, while 47 children served as controls. No subsequent differences were observed in their antibody responses to tetanus toxoid (114). Several groups have observed an enhanced antibody response and increased numbers of splenic plaque-forming cells in animals given a large dose of vitamin A. Mice showed increases in both primary and secondary immune responses to tetanus toxoid after receiving a single massive dose of vitamin A (114). Two daily ip injections of vitamin A in mice were followed by a large increase in splenic plaque-forming cell numbers after inoculation with sheep RBC and an increased antibody response to a dinitrophenol-ovalbumin hapten (115). Daily injections of vitamin A in mice for 5 days before, or after, administration of sheep RBC led to increased HA antibody production and splenic plaque-forming cell numbers (116).

3. Cell-mediated immunity. Vitamin A also influences cell-mediated immunity. Splenic lymphocytes from vitamin A-deficient rats showed a 33% reduction in in vitro transformation responses after PHA, Con A, or LPS stimulation (106). Supplementation of the deficient rats with vitamin A caused transformation responses to normalize within a 3-day period. The in vitro addition of vitamin A in either an aqueous or oily form led to dose-dependent enhancement of antigen stimulated proliferation of normal human lymphocytes (117). A markedly diminished uptake of radiolabeled thymidine by rat thymic and splenic lymphocytes, as reported by Krishnan et al. (113), may have been due, in part, to coexisting PEM.

Bhaskaram and Reddy (118) reported a normal *in vitro* response to PHA by blood lymphocytes obtained from vitamin A-deficient children, but diminished DDH responses to PHA were seen in four of nine children with vitamin A deficiency. Brown et al. (114) did not find differences in DDH responses to PPD in children given a single large dose of vitamin A in comparison to responses of a control group. In contrast, in a prospective study, alternate preoperative patients were begun on a 7-day course of vitamin A therapy, receiving 30 to 45 $\times$ 10^4 IU/day (119); these individuals did not exhibit the postoperative depression of lymphocyte counts or responsiveness in mixed lymphocyte cultures as seen in the untreated control group.

The acute administration of a large dose of vitamin A to sensitized guinea pigs diminished the magnitude of DDH and Arthus reactivity during skin tests with diphtheria toxoid (120). It was postulated that the acute excess of vitamin A had suppressed the inflammatory response.

Jurin and Tannock (116) injected female C57BL/6 mice with vitamin A for 5 consecutive days before or after grafting with isologous male skin. The administration of vitamin A caused a more rapid rejection of these grafts.

4. Other effects. A series of four daily injections of 3000 IU vitamin A in mice caused no change in RES clearances of colloidal carbon or aggregated albumin (112). Krishnan et al. (113) noted a diminished number of glass-adhering cells in peritoneal exudates from vitamin A-deficient rats.

Madjid et al. (121) reported an increase in the concentration of hemolytic complement of rats given a vitamin A-deficient diet; forced feedings were used to maintain a full protein intake, since PEM is associated with low complement values.

In addition to the role of vitamin A as an essential nutrient, this vitamin serves as an adjuvant if mixed with antigens before their inoculation (113, 122).

5. Comments. Because of the high incidence of human deficiencies in certain areas of the world, vitamin A is a highly important individual micronutrient. Isolated deficien-

cies can occur in the absence of PEM. Although a considerable body of data attests to the importance of vitamin A in host resistance, there is a paucity of studies dealing with direct immune systems interactions. Available reports do, however, point toward effects of vitamin A on humoral and cell-mediated immune mechanisms, but almost no work has dealt with phagocytic cell functions. Only the most rudimentary of clinical investigations have been conducted on immune system competence of vitamin A-deficient human populations. Both basic and clinical areas of vitamin A-defiency need modern investigation. The growing use of large doses of this vitamin by food-fadists may call for studies as well on the immunological effects of hypervitaminosis A.

B. Vitamin E (α-tocopherol)

Vitamin E is believed to serve as an antioxidant, to scavenge free radicals, to stabilize cellular membranes, and to have beneficial effects with respect to fertility and aging (123–129). For these reasons, vitamin E is one of the nutrients some individuals elect to consume in megadose quantities.

1. Host resistance. Recent reports (123–127, 130–138) described studies in both laboratory and farm animals suggesting that doses of vitamin E, somewhat in excess of those generally recommended, may increase immune responses to antigens and improve host resistance against challenge with microorganisms.

In this regard, a 3- to 6-fold increase in the dietary intake of vitamin E, achieved by providing 150 to 300 mg/kg in feed, increased the resistance of chickens against experimental *Escherichia coli* infection (123–125). In mice previously immunized with a pneumococcal polysaccharide vaccine, addition to the diet of 180 mg *dl*-α-tocopherol acetate per kg feed increased the survival rate from 20 to 80% after challenge with either a small or large inoculum of virulent type-1 pneumococcus (124, 130). Nockels (127) showed that vitamin E-supplemented chickens and turkeys had an increased resistance to induced *E. coli* infections, and that the ability of sheep to resist chlamydial infections was increased if they were fed a diet containing a modestly increased quantity of vitamin E.

Ayres and Mihan (130) described clinical studies in which large daily doses (800 to 1600 IU) of potent vitamin E preparations were given to patients with scleroderma, discoid lupus erythematosus, porphyria cutanea tarda, polymyositis, and some forms of vasculitis. Since no control studies were done, claims that these diseases were brought into remission lack scientific validity.

2. Humoral immunity. Independent investigations consistently report that the addition of moderate amounts of vitamin E to the feed of animals will heighten humoral responses to vaccines. Barber et al. (131) showed that the intramuscular (im) administration of *dl*-α-tocopherol (33 IU/kg) to guinea pigs before and after the administration of a living attenuated TC-83 strain Venezuelan equine encephalomyelitis vaccine led to higher HAI titers; such an increase did not occur if the same amount of vitamin E was given orally. Ellis and Vorhies (132) fed 6- to 8-wk-old pigs a control diet or diets containing daily excesses of 20,000 or 100,000 IU vitamin E. After immunization with formalinized *E. coli*, the group given the most vitamin E developed primary HA titer rises 2-to 3-fold higher than the other groups; however, all groups showed an equal response to a booster injection of the same vaccine. Harman et al. (133) added vitamin E (0.25% by weight) to the diet of mice during a study of aging which lasted from the 6th to the 88th wk of life. These mice showed an increased humoral response and greater numbers of splenic plaque-forming cells after the administration of sheep RBC. Heinzerling et al. (123) showed that the HA antibody response of chicks was increased 2- to 3-fold against sheep RBC if they were fed a 3- to 6-fold excess of dietary vitamin E; similarly, mice fed an increased quantity of vitamin E showed increased humoral responses to sheep RBC or tetanus toxoid (124). Nockels (127) reviewed studies of dietary vitamin E supplementation along with her own work and observed that supplemented mice given sheep RBC or tetanus toxoid also responded with increased numbers of plaque-forming cells in addition to the increased HA antibody titers responses; in contrast, plaque-forming cell numbers were decreased and no humoral response was detected in mice whose diet was deficient in vitamin E. In comparison to controls, hens fed an excess of vitamin E developed higher HA titers during *E. coli* infections and became capable of passing on humoral anti-

bodies from *Brucella abortus* vaccine to their chicks (127).

3. Other effects of vitamin E excess. Evidence for the effect of vitamin E supplementation on other immune system functions is presently limited to single reports. Campbell et al. (134) solubilized *dl*-α-tocopherol and tocopherol acetate with emulsifying agents and added it in vitro to mouse spleen cells. The in vitro production of antibodies against sheep RBC normally requires the presence of both adherent and nonadherent spleen cells, but the addition of vitamin E allowed antibody production to occur in the absence of adherent cells. Cultures that received in vitro vitamin E showed increased titers (134).

Vitamin E stimulates mitogenic reactions in murine spleen cells. Corwin and Shloss (135) showed that at suboptimal vitamin concentrations, vitamin E stimulated mouse splenic T-lymphocyte responses to low levels of the mitogen, Con A. This did not occur when Con A was itself at optimal concentrations. Vitamin E was most stimulatory when dietary polyunsaturated fatty acids (PUFA) were low. A requirement for thymic factors for permitting vitamin E to stimulate mitogenesis of B-cells was suggested by the observation that vitamin E could stimulate responses to LPS in lymphocytes from normal mice but not in those from athymic, nude mice (135).

Heinzerling et al. (126) reported that the administration of vitamin E to mice by diets containing 180 mg/kg increased the rate of RES clearance of carbon from the blood stream. When supplemented mice were immunized with a pneumococcal vaccine, their white cells had a 4-fold greater increase in phagocytic index than cells from immunized controls which did not receive extra dietary vitamin E.

Likoff et al. (125) and Tengerdy et al. (124) observed that prostaglandin synthesis was diminished in bursa and spleen cells of chicks fed a diet containing 300 ppm of vitamin E. Since increased local concentrations of prostaglandins may be immunosuppressive (136, 137), it was postulated that vitamin E was beneficial in host defenses by preventing the infection-induced increases in tissue prostaglandins through its abilities to inhibit synthesis of these substances (137).

4. Synergism with selenium. Several studies suggest that the immunostimulatory effects of vitamin E are magnified if administered concomitantly with modest excesses of selenium. Heinzerling et al. (123) reported that combined supplementation with both vitamin E and selenium increased the humoral response of mice against sheep RBC and tetanus toxoid. On the other hand, a diet deficient in both vitamin E and selenium led to reduced plasma glutathione peroxidase activities. Activity could be restored by adding either vitamin E or selenium back to the diet (7); selenium is a component of one form of this metalloenzyme. Noguchi et al. (139) suggested that vitamin E functions within the lipid membranes of cells by neutralizing free radicals as well as by its antioxidant effects. Despite these new data indicating that vitamin E, selenium, or both in combination, can influence glutathione peroxidase activity, no study has been reported in which these essential micronutrients have been used in an attempt to reverse the inherited deficiency of this enzyme in the neutrophils of a subset of patients with familial chronic granulomatous disease.

5. Harmful effects of excess vitamin E. In contrast to reports suggesting that an increased intake of vitamin E was beneficial, Prasad (140) gave megadose quantities of vitamin E (300 ml/day *dl*-α-tocopherol acetate) to 18 healthy volunteer subjects for 21 consecutive days. Diminished in vitro bactericidal activity of neutrophils and responsiveness of lymphocytes to PHA were found at that time; however, when PHA was used for skin testing these subjects (140), DDH reactions were unchanged.

6. Effects of vitamin E deficiency. Hamilton et al. (141) reported that rabbits with an induced deficiency of vitamin E had a 10-fold reduction in the number of peritoneal cells accumulating 48 h after ip mineral oil. However, vitamin E deficiency did not alter cellular chemotactic activity.

Warshauer et al. (142) noted that a vitamin E deficiency increased the susceptibility of rats of ozone-induced pulmonary damage. Bactericidal activity of pulmonary macrophages was decreased after a 1-wk exposure of vitamin E-deficient rats to ozone.

7. Comments. These studies on vitamin E are unlike those of most other essential micronutrients, in that they are chiefly con-

cerned with the effects of administered excesses rather than those of naturally occurring or experimentally induced deficits. The evidence seems clear, however, that the administration of α-tocopherol in amounts somewhat in excess of minimal recommended doses has an often beneficial effect on host resistance in a variety of test animals. Further, the immune system appears to contribute to this enhancement of resistance. Although such benefit has not been demonstrated by any scientifically valid study in man, an important cautionary note has been introduced by Prasad's recent findings (140) concerning the multifaceted nature of immunosuppressive effects of megadose vitamin E administration to volunteers. Questions raised to date certainly call for an increase in both basic and clinical studies of vitamin E interactions with the immune system.

IV. Amino acids

Although numerous observations attest to the importance of adequate protein nutrition for maintaining immune system competence, few studies have attempted to determine if single amino acids have an individual role. Isolated deficits or excesses of a single essential amino acid, or an imbalance among essential amino acids, do seem to be reflected at least by functional changes in humoral immunity. Continued deficiencies of branched-chain or sulfur-containing amino acids lead to a depletion of cells from lymphoid tissues (9). Deficiencies or imbalances of single amino acids may impair the production of antibodies in response to various test antigens. Studies concerning the effect of such amino acid abnormalities on cell-mediated immunity are few (143). Neither positive nor negative data exist related to possible effects of single nonessential amino acids on immune system function.

A. Essential amino acids

As early as 1951, Ludovici and Axelrod (18) demonstrated that a combined deficiency of tryptophan and niacin in rats led to depressed HA antibody response to immunization with human RBC. Kenney et al. (144) showed that tryptophan-deficient diets in rats led to depressed hemolysin titers of IgG and IgM antibodies after immunization with sheep RBC. This depression could be reversed by restoring tryptophan to the diet. Gershoff et al. (27) reported that tryptophan deficiency impaired the antibody response of rats to sheep RBC or to synthetic poly-amino acid antigens administered in complete Freund's adjuvant. Jose and Good (143) showed that moderately severe deficiencies of tryptophan in mice were associated with depressed HA and blocking antibody responses when the deficient mice were inoculated with mammary carcinoma cells. In contrast, these mice developed normal cytotoxic spleen cell activity against the tumor cells. A combined deficiency of phenylalanine and tryptophan led to partial atrophy of the spleen and thymus and to an impaired ability of the RES to clear radioiodinated polyvinyl pyrrolidone (145).

An isolated phenylalanine deficiency in rats also reduced the ability to generate a normal antibody response against sheep RBC cells or against synthetic peptides in complete Freund's adjuvant (27). A combined deficiency of phenylalanine and tyrosine in rats profoundly depressed HA and blocking antibody responses to inoculated tumor cells without influencing the cytotoxic cell-mediated immune response (143). Similar immune deficiencies resulted from moderate individual deficiencies of valine, threonine, or isoleucine, or a combined deficiency of methionine and cystine. Isolated deficiencies of arginine, histidine or lysine produced moderate impairment of the HA or blocking antibody responses to tumor cell antigens. An isolated lysine deficiency had no apparent effect on the production of hemolysin antibodies by rats inoculated with sheep RBC (144).

B. Branched-chain amino acids

Isolated dietary deficiency of a single branched-chain amino acid, initiated in mice soon after weaning, led to impaired resistance and antibody response against *S. typhimurium* but had no detectable effect on cell mediated immunity (146). A moderate deficiency of leucine in rats had no discernible effect on the production of HA or blocking antibodies (143), but in contrast to all of the other amino acid deficiencies or imbalances, leucine deficiency increased the activity of cytotoxic

spleen cells directed against inoculated tumor cell antigens.

Chevalier and Aschkenasy (147) found that a 7% dietary content of leucine did not adversely influence the immune response of injected sheep RBC in young rats. However, a similar 7% dietary overload of leucine in rats fed a protein-poor (4% casein) diet had the effect of reducing the HA and hemolysin antibody responses as well as splenic plaque-forming and T-rosetting cell numbers. The combined effects of a leucine overload with a protein-poor diet was as great as that which could be produced by complete removal of protein from the diet. The combination of a 7% dietary leucine overload in rats fed a 4% casein diet also produced deficiencies in body growth and lymphopoiesis, and markedly diminished serum IgG concentrations (148). Interestingly, immune system effects of this imbalance were prevented by adding small (0.2%) amounts of both valine and isoleucine to the rats given the 7% leucine overload.

C. Lipotropic amino acids

Methionine and choline have been grouped with folic acid and vitamine B_{12} under the category of lipotropic factors. Forty years ago Griffith and Wade (149) found thymic involution in young rats fed a choline-deficient diet. Thymic regeneration followed refeeding with choline. As noted earlier, Jose and Good (143) demonstrated a profound decrease in antibody response in rats with a combined deficiency of methionine and cystine.

Williams et al. (150, 151) studied immune functions in adult rats marginally deprived of methionine and choline during intrauterine development and/or the preweaning period, but thereafter given a complete diet. Adult rats, whose mothers had been deprived of methionine and choline during gestation, showed a depressed primary response to sheep RBC. Adult rats whose mothers had been deprived of these amino acids only during lactation showed a depressed splenic lymphocyte response when stimulated in vitro by Con A or allogeneic lymphocytes. Their DDH responses to PHA were also depressed. Adult rats from dams subjected to deprivation of methionine and choline during both gestation and lactation showed all of these abnormalities plus depressed in vitro stimulation of their thymic cells by pokeweed mitogen and depressed resistance to infection with *Salmonella typhimurium* (151).

Newberne and colleagues (152–155) also studied rats fed one of four different intake levels of dietary lipotrophic factors. Rat pups bred from mothers with the greatest gestational deprivations were highly sensitive to *S. typhimurium* infections, even after they became adults. This residual decrease in resistance was not corrected by feeding the growing rats a complete diet from the age of weaning. Further, these adult rats had a diminished RES mass (154, 155) as a residual effect of lipotropic factor deficiency during gestation.

Mice fed a diet deficient in both choline and B_{12} maintained normal growth and normal percentages of both T- and B-lymphocytes in peripheral blood (15). However, the mice exhibited a marked decrease in the in vitro response of splenic lymphocytes to PHA and LPS and diminished splenic plaque-forming cell production after immunization with sheep RBC. In contrast, these deficient mice showed normal HA antibody titers after sheep RBC and an increased, rather than a decreased, response of splenic lymphocytes to the addition of mitomycin-blocked allogeneic lymphocytes in mixed cell cultures (150).

Gershoff et al. (27) could not demonstrate that an increase or decrease of methionine in the diets of rats had any effect on their antibody responses to either sheep RBC or a synthetic polypeptide antigen. However, the use of a methionine antagonist, ethionine, diminished the immune response to these antigens. No deficiency in the primary immune response occurred in cebus monkeys fed a diet either deficient or excessive in methionine, or during ethionine administration, but diminished secondary antibody responses were found (156).

D. Comments. The ability of body cells to synthesize new proteins is a key necessity for developing and maintaining humoral and cell-mediated immunity and for assuring optimal function of other host defensive measures (157). The regulation and adequacy of immunological functions in the host ultimately depends upon, or is influenced by, the availability of free amino acids to different cell populations of the body. The movement of plasma free amino acids into or out of body cells has been studied in greatest detail

in liver and muscle. Little is known about the ability of lymphocytes, plasmacytes, or phagocytes to compete with other cells for amino acids present in plasma.

In patients with kwashiorkor or PEM, the concentrations of individual plasma free amino acids become depressed to varying degrees. The severity of hypoaminoacidemia correlates well for most individual amino acids (especially the essential ones) with the magnitude of serum albumin depression. Studies with radioactively tagged amino acids have shown that rats respond to an infection with an increase in the synthesis of immunoglobulins even though their concomitant production of serum albumin is markedly retarded (158). These observations suggest that some mechanism must exist to prioritize the cellular use of amino acids in order to favor host survival (157). In underdeveloped countries, children with kwashiorkor or PEM often show normal-to-high immunoglobulin concentrations and increased IgG synthesis, even though their ability to respond to new antigens is impaired. This paradox may be explained by the massive and continuous "antigenic pressure" arising from chronic parasitism plus repeated and prolonged infectious episodes in these children. Despite their lack of body protein, these children appear able to divert sufficient amino acids from other potential uses to manufacture plasma immunoglobulins and acute-phase reactant proteins.

The insights provided by animal studies of isolated single (or dual) essential amino acid deficits, or the imbalance created by a dietary leucine excess, are dissimilar from insights obtained during clinical studies in malnourished children. Complicating infections or severe inanition are not generally present in the animals used for relatively brief experimental studies of an isolated amino acid deficiency or excess. Accordingly, the most prominent change observed in animal studies is a partial deficit in the primary de novo production of antibody directed against a new antigen. This relatively subtle immune dysfunction could be due simply to the lack of a suitable distribution of amino acids required for protein synthesis by a new clone of antibody-producing cells. Protein synthesis by these newly-formed cells is the final step in a long and complex immunogenic sequence. Antigen recognition and processing, and repeated cellular transformations must occur before the new clones of antibody-producing plasma cells are created, but only the final production of a new immunoglobulin seems deficient in animals subjected to brief periods of amino acid imbalance.

Greater or more lasting amino acid deficits could be expected to produce progressively more serious defects in immune function, deficient splenic plaque-forming cell numbers, deficient T-lymphocyte numbers and responsiveness, and eventually lymphoid tissue atrophy. All of these problems are seen in chronically malnourished children.

V. Lipids

The possibility that individual dietary lipids could influence host immunological defenses was first suggested by Dewey and Nuzum (159) who demonstrated suppressive effect of cholesterol injections in guinea pigs and rabbits on the phagocytic function of circulating granulocytes. Subsequent studies have produced data concerning the discrete roles of cholesterol, total lipids, and both saturated and polyunsaturated fatty acids on host immune responsiveness and nonspecific defenses against infectious illnesses (see **Table 5**).

Virtually no information is available concerning the effect of dietary phospholipids on immune functions. However, the methylation of cellular phospholipids is stimulated by lectins, immunoglobulins, and chemotactic proteins (160). This causes the translocation of the phospholipids from the inside to the outside of cellular membranes and facilitates mitogenesis in lymphocytes, chemotaxis in neutrophils, and the release of histamine from mast cells and basophils. Alterations in cell membrane fluidity are caused not only by the translocation of phospholipids, but also by differences in the amount of cholesterol or PUFA present in the lipid bilayer. These lipids may influence immune functions by changing cell membrane fluidity.

In addition to the immunological effects of lipid nutrients when consumed in the diet or injected parenterally in large quantities, some lipids can be used as adjuvants if they are combined with injected antigens (161). Artificially created lipid micelles also have poten-

TABLE 5
Interaction of lipids with immune system functions

	High fat intake and/or obesity	Polyunsaturated fatty acid deficit	Polyunsaturated fatty acid excess	Cholesterol excess
Host susceptibility to infection	Increased			Increased
Lymphoid tissues	Relative decrease in size		Thymic and splenic weight loss	
In vitro lymphocyte responses	Altered surface membrane fluidity		Suppressed response to mitogens. Altered killer cell activity	Diminished cytotoxic activity Diminished response to mitogens
Serum immunoglobulin concentrations		Some may increase		
Antibody production		Impaired		Variable; may be suppressed
Splenic plaque cells		Diminished production		
Allograft survival		Shortened	Prolonged	
Delayed dermal hypersensitivity				Suppressed
Experimental allergic encephalitis		Increased susceptibility	Decreased susceptibility	
Neutrophil chemotaxis	May be slowed			
Neutrophil phagocytic activity	May be depressed			
Neutrophil bactericidal activity	May be depressed			
Macrophages		May alter motility		Foam cell appearance
Reticuloendothelial system	Clearance slowed		Clearance accelerated	Effects inconsistent
Other				Oxidized metabolites may be immunosuppressive

tial immunogenic value if various types of antigens are included within their interior lamellar spaces. These micelles can then be used, via inoculation, in an effort to deliver drugs or antigens into the protoplasm of single cells within the body. The recipient host cells must be capable of taking up the micelles from the circulation via phagocytic processes. Since this work with lipid adjuvants and micelles is pharmacological in concept, it will not be considered further in this review which is limited solely to the immunological effects of the lipids used as nutrients.

A. Total lipids

Alterations in the total body content of fat have received relatively little attention in terms of a specific impact on discrete aspects of immune function.

1. Host resistance. Newberne (162) showed that obese beagle dogs developed paralytic encephalitis of increased severity when inoculated with distemper virus in comparison to the responses of normally nourished or slightly underfed litter-mate controls. The three groups showed no differences in their serum IgG responses to this infection. Fiser et al. (163) found that experimentally induced illness after inoculation with infectious canine hepatitis virus was more severe and longer lasting in beagle pups fed a high-fat ration than in litter-mates fed a normal diet.

In contrast, Hedgecock (164) showed that the use of a saturated vegetable oil (i.e., coconut oil) as the sole source of diet fat increased the resistance of mice to experimental tuberculosis. He was also able to induce this increase in resistance by feeding the mice with a synthetic mixture of fats, including methyl aurate, mysterate, and palmitate designed to reproduce the composition of coconut oil. Uncertainties about the role of

dietary fat on resistance are illustrated further by a study (165) in which an isocaloric substitution of dietary carbohydrate by fat in rats led to increased survival rates after infection with *Pasteurella multocida*, decreased infection rates after exposure to *Fasciola hepatica* or *Ascaris suum*, and a lower incidence of hepatic tumors induced by 4-dimethylaminoazobenzene.

Genetically obese mice, C57BL/6J (ob/ob) exhibit a variety of immunological abnormalities that could predispose them to infectious illness (166). Their thymic and splenic tissues are small in size and contain fewer than normal numbers of mononuclear cells. Although the number of splenic IgG-producing cells is diminished, there is a slight increase in antibody-dependent cell-mediated cytotoxicity of spleen cells and a marked increase in the number of killer T-cells, as assayed by ^{51}Cr-release studies.

2. RES function. Several groups have demonstrated that the concentration of lipids in serum can influence the ability of RES cells to clear particulate substances from blood. Di Luzio and Wooles (167) showed that an iv injection of methyl palmitate in mice caused a defect in the ability of Kupffer cells to perform phagocytic functions. An antigenic challenge of these mice with sheep RBC was not followed by the normally expected production of HA antibodies. Berkin and Benacerraf (168) reported that an iv infusion of ethyl stearate in mice inhibited the clearance of colloidal carbon while an iv injection of glycerol trioleate increased clearance values. In contrast, a large oral load of these lipids, or of olive oil, caused a decrease in RES clearance functions which lasted from 1 to 2 days.

3. Single cell effects. An iv infusion of soy bean oil emulsion in healthy subjects caused a dose-related inhibition of the random activity and chemotactic responsiveness of neutrophils which persisted for almost a day. Similar changes could also be induced by adding the soy bean oil emulsion in vitro to isolated neutrophils (169).

Wardle (170) suggested that fatty acids could alter the distribution of membrane receptor groups on lymphocyte surfaces by actions involving enzyme induction, prostaglandin synthesis, or solely by their detergent effect. Other possibilities include the actual incorporation of cholesterol and fatty acids with different degrees of unsaturation into the lipid bilayer of the external cellular membranes. Since fatty acid carbon chains have an angular bend at the site of each unsaturated double bond, their insertion into a lipid bilayer requires more space than does the straight chains of saturated fatty acids. Their insertion could thus result in alterations of membrane fluidity as well as in the ability of receptor sites to be translocated during lymphocyte capping, a process believed to require surface membrane participation.

Hawley and Gordon (171) studied the chemotactic, phagocytic, and bactericidal activities of human neutrophils incubated in vitro with different free fatty acids bound to albumin. Palmitic acid (C16:0), added at high in vitro concentration, caused an almost complete cessation of chemotactic activities and a moderate inhibition of phagocytic and bactericidal activity; smaller concentrations caused only a modest inhibition of chemotaxis. Very high concentrations of oleic acid (C18:1) caused a slight diminution in phagocytic and chemotactic activity. Both fatty acids produced structural changes when the incubated neutrophils were visualized microscopically. These observations suggested that the surface membranes of phagocytic cells as well as lymphocytes were influenced by the variety and concentration of individual free fatty acids in their surrounding environment.

B. Polyunsaturated fatty acids

The concept that individual fatty acids may influence discrete immune functions is strengthened by recent observations that the physical structure of individual fatty acids is of marked importance. The presence of unsaturated bonds is perhaps the most crucial factor. In this regard, studies with oleic acid (C18:1), with its single double bond, clearly differentiate it from all of the saturated fatty acids with chain lengths from 12 to 18. Increases to 2, 3, or 4 double bonds in a single molecule have a progressively greater impact upon an immune function under study, as exemplified primarily by studies with linoleic acid (C18:2), linolenic acid (C18:3), and arachidonic acid (C20:4).

1. Lymphoid tissue effects. Mertin et al. (1972) showed that the administration of linoleic acid to CBA mice, orally or by sc injec-

tion, caused a loss of thymic weight and an enlargement of peripheral lymph nodes. The RES of these mice showed an enhanced capacity for clearing colloidal carbon. Erickson et al. (173) found that an increased concentration of PUFA in the diet of growing mice led, at times, to a decrease in splenic and thymic weights. Fatty acids capable of being metabolized to arachidonic acid have the greatest thymolytic activity (174). Thymolysis is induced by arachidonic acid to an equal degree in intact or adrenalectomized mice, but this effect is blocked by drugs which prevent the conversion of arachidonic acid to prostaglandin (174). Stuart et al. (175) used injections of various lipids to study in vivo effects on the RES. Olive oil or glycerol trioleate markedly enhanced RES clearance of carbon, whereas glycerol monooleate was without effect, and ethyl stearate or ethyl oleate were both markedly inhibitory at high concentrations.

2. Humoral responses. A deficiency of essential PUFA can be deleterious. DeWille et al. (176) fed mice for 28 days on diets that lacked PUFA. Although no detectable effect was evident on the growth or appearance of the mice, both the IgM and IgG responses were inhibited after the administration of sheep RBC and there was a diminished appearance of splenic plaque-forming cells. A second inoculation with sheep RBC 28 days after the first demonstrated a reduced memory response in the PUFA-deficient mice. Immunization with LPS, a T-cell independent antigen, also led to diminished formation of splenic plaque-forming cells. All depressed functions were corrected within 7 days after PUFA were restored to the mouse diets (176). Concentrations of IgG_1 and IgG_2, but not IgA or IgM, increase in mice fed a high PUFA diet in comparison to findings in controls (173).

3. Cell-mediated immunity. Although splenic natural killer cell activity was not diminished in rats fed a 20% PUFA diet, nearly total suppression of the proliferative splenic T-cell response to PHA was observed; a PUFA deficiency intensified this response (177). Other evidence for the role of PUFA in cell-mediated immune functions comes from experiments dealing with the production of experimental allergic encephalomyelitis and studies of skin graft survival.

Because myelin contains relatively high amounts of PUFA, Clausen and Møller (178) used rats that had been bred and raised on a diet deficient in PUFA to determine if susceptibility to the experimental induction of allergic encephalomyelitis would be altered. This cell-mediated immunological process was produced by the sc injection of homogenized guinea pig brain in complete Freund's adjuvant. PUFA-deficient rats were more susceptible than control rats fed a normal diet. To test the opposite effect, Selivonchick and Johnston (179) studied pregnant rats and their progeny in groups fed control, low-fat, or low-fat-plus-linoleic acid diets. The induction of experimental allergic encephalomyelitis occurred most easily in rats bred and raised on the low fat diet, whereas the linoleic acid supplemented diet was protective. Weston and Johnson (180) also observed that rats fed a PUFA-deficient diet developed a higher incidence of experimental allergic encephalomyelitis than controls. However, Levine and Sowinski (181) attributed any alterations in the severity of allergic encephalitis to thymic atrophy and nonspecific stress rather than to PUFA availability.

Ring et al. (182) first showed that skin allografts survived twice as long on white Sprague-Dawley rats as control grafts if linoleic acid was added to the diet for 15 days after grafting or given ip at the time of grafting. However, the ip injections caused high mortality rates.

The injection of 20 μg of linoleic acid (sc, three times per week) in female CBA mice prolonged skin graft survival against a strong histocompatibility barrier and blocked susceptibility of injected mice to experimentally induced allergic encephalomyelitis (183–186). Dietary administration of linoleic acid also increased the survival of skin allografts in mice. In contrast, PUFA deficiency shortened skin allograft survival. Meade and Mertin (187) also found that sc PUFA, especially linoleic acid, prolonged the survival of skin allografts in mice and inhibited the cytotoxic response of allogeneic spleen cells. PUFA inoculations led to an increase in spleen and lymph node weights, decreased the number of spleen cells exhibiting Thy-1.2 surface antigen, and eventually led to destructive changes within the spleen. In reviewing the effects of PUFA on graft prolongation,

Hughes et al. (188) stressed the critical importance of the dose, composition, and route of PUFA administration as well as the degree of histocompatibility differences.

In a study performed in man, Uldall et al. (189) and McHugh et al. (190) used double-blind control diets in 89 patients who had received a cadaver kidney transplant. Forty-four individuals received excess dietary PUFA and 45 were given an oleic acid placebo. All other immunosuppressive measures were standardized in an attempt to make the two groups uniform. The functional aspects of graft survival appeared better in the PUFA-fed subjects for a period of 3 to 4 months, but between-group differences could no longer be detected after 6 months. Complications in the two groups were assessed as being equal.

When mice were given linoleic or oleic acid by direct infusion into the duodenum, Frost et al. (191) were unable to detect any differences in rates of lymph flow from the thoracic duct, or in the number or responsiveness of duct lymphocytes to PHA or Con A. Subcutaneous injections of CBA mice with 20 μg of linoleic acid three times a week inhibited lymphocyte proliferative responsiveness to the mitogen PHA or to the antigen PPD in antigenic doses (184). Mertin (183) also observed that linoleic acid injections blocked the primary and secondary cytotoxic responsiveness in mice. Meade and Martin (187) noted a diminished cytotoxic response by spleen cells isolated from mice treated with sc linoleic acid injections.

4. Single cell effects. A limited number of studies suggest that PUFA can affect in vitro macrophage functions. The electrophoretic mobility of human macrophages was altered by the in vitro addition of PUFA (186). PPD slowed the in vitro mobility of normal macrophages, but when oleic, linoleic, or arachidonic acids were added, the latter two unsaturated fatty acids were found to inhibit PPD-induced mobility of macrophages in all in vitro tests.

The inability of lymphocytes from patients with multiple sclerosis to agglutinate in vitro in the presence of Con A or measles antigen, as do normal human lymphocytes, was corrected by long-term dietary linoleate feedings given as safflower oil (192). Lymphocytes from patients with multiple sclerosis were much more sensitive than those from normal subjects to the inhibitory action of linoleic acid (0.08 mg/ml) when tested in vitro (193); macrophage mobility stimulated with thyroglobulin was also diminished by approximately 90% after the addition of linoleic acid. Strunk et al. (194) showed that the addition of soy oil emulsions in vitro to guinea pig peritoneal macrophages diminished their ability to "spread" on glass, to form typical ruffles, or to phagocytize latex beads, yeast organisms, or IgG-coated sheep RBC. Field and Shenton (136) added linoleic acid to macrophages and lymphocytes and observed that it inhibited PPD-stimulated mobility within seconds. The interpretation of all such in vitro studies has been questioned. Frost et al. (195) found that PUFA are toxic if added directly to lymphocyte cultures in vitro and cautioned that any in vitro addition of PUFA must consider toxic effects as a possible explanation for observed results. Similarly, Tonkin and Brostoff (196) found that PUFA, when bound to albumin as they are in vivo, did not inhibit lymphocyte transformation. They argued that the addition of PUFA dissolved in alcohol to cell cultures was nonphysiological. Other studies suggest that cultural conditions are of major importance (189). The incubation of human lymphocytes with oleic or linolenic acids altered the unsaturated-to-saturated ratios of cell membrane phospholipids but produced only slight enhancement of natural killer cell activity (190). Feeding of diets deficient in essential PUFA can markedly alter the lymphocyte content of PUFA (191) but with relatively little effect on their mitogenic responses.

Offner and Clausen (200) studied the uptake of myo-[2-^{3}H]inosotol into phosphatidylinosotol of human lymphocytes as induced with PPD or PHA in the presence of 0.08 mg/ml concentrations of linoleic, arachidonic, or oleic acids, or the prostaglandins E_1 and E_2. Although all substances were inhibitory, both linoleic and arachidonic acids showed the greatest inhibition of uptake as stimulated either by the mitogen or the antigen. In a subsequent study (201), addition of the same quantity of linoleic acid to the lymphocytes of 16 normal individuals increased the percentage of T-lymphocytes showing both total and avid rosette formation. Smith et al. (202) incubated human blood or rat

spleen lymphocytes with free fatty acids in vitro and studied the effects of stimulation by the mitogen PHA in 0.1 to 0.2 μM concentrations; linoleic acid was suppressive and heptadecanoic acid (C17:0) was markedly suppressive. Kelly and Parker (203) found that 0.1 to 5.0 μg/ml of arachidonic acid, in media supplemented with fetal calf serum enhanced thymidine uptake by PHA-stimulated human leukocytes. In contrast, 240 μg of linoleic, oleic, or arachidonic acid dissolved in alcohol were inhibitory (219). On the other hand, Mihas et al. (204) reported that synthetic 16,16-PGE_2 and PUFA dissolved in alcohol would inhibit lymphocyte transformation reactions, but without any evidence of cytotoxicity.

Finally, Klausner et al. (205) studied the capping of Ig receptors in mouse B-lymphocytes; 2 to 4 M/100 ml concentrations of *cis*-unsaturated fatty acids inhibited lymphocyte capping but *trans*-, unsaturated or saturated, FFA did not. The inhibition effect on capping was reversible in the presence of calcium ions but not in the presence of magnesium ions. It was suggested (205) that a calcium-linked binding protein served as an intramembraneous anchor to immobilize cross-linked receptors and that the *cis*-unsaturated FFA could penetrate the lipid domains associated with the anchoring protein and interrupt their linkage to the cytoskeleton.

C. Cholesterol

All studies concerning the immunological effects of cholesterol as a single nutrient have dealt with hypercholesterolemic states. These have been achieved either by the manipulation of experimental diets or by the direct inoculation of cholesterol into experimental animals.

1. Host resistance. Cholesterol injections prolonged survival of guinea pigs during acute infections with highly virulent tubercle bacilli, but were without effect during chronic tuberculosis or during infections caused by strains of lesser virulence (206). Fiser et al. (207) found that experimentally induced chronic hypercholesterolemia neither increased nor decreased the ability of rhesus monkeys to withstand an induced pneumococcal infection successfully.

Increased concentrations of plasma cholesterol may have a deleterious effect on host resistance (208). A high cholesterol, atherogenic diet caused normally resistant rats to become highly susceptible to tuberculosis (209). After hypercholesterolemia was induced by dietary manipulation in mice infected experimentally with a human cardiotrophic Coxsackie virus B5, virus titers increased and persisted in aortic tissues and there was a marked increase in cardiomyolysis and damage to the aorta in comparison to findings in similarly infected control mice (210). Kos et al. (211) also observed an increase in the susceptibility of hypercholesterolemic mice to Coxsackie virus B5 or *L. monocytogenes* infections; the ability of *Corynebacterium parvum* infections to inhibit the formation of induced fibrosarcoma was also reduced in hypercholesterolemic mice. Induced hypercholesterolemia diminished the resistance of rats to dimethyl hydrazine-induced tumors (177).

2. Lymphoid tissue effects. No studies have reported a direct association of hypercholesterolemia with altered lymphoid tissue histology. However, peritoneal macrophages obtained from hypercholesterolemic rabbits showed an increased content of cholesterol and cholesterol esters, and some macrophages resembled foam cells in appearance (212, 213). Despite these changes, the macrophages could still destroy tubercle bacilli (212).

3. Humoral immunity. The induction of hypercholesterolemia by dietary measures has led to divergent findings concerning effects on humoral and cell-mediated immunity. In comparison to findings in controls, hypercholesterolemic rabbits developed increased antibody titers in response to immunization with typhoid vaccine (208, 214), but diminished HA and precipitin antibody responses when inoculated with ovalbumin in complete Freund's adjuvant (207). Hypercholesterolemic mice showed a diminished HA response to inoculation with sheep RBC in comparison to responses of control mice (211). After the induction of hypercholesterolemia, rhesus monkeys developed HA antibodies against a primary immunization with ovalbumin in a normal manner, but the appearance of precipitin antibodies after a booster inoculation was delayed and decreased in comparison to that of controls (207).

4. Cell-mediated immunity. Hypercholes-

terolemic monkeys demonstrated an increase in DDH response to PPD skin testing following an inoculation of antigen contained in complete Freund's adjuvant (207). Hypercholesterolemic rabbits also showed increased responsiveness after being sensitized with BCG vaccine (208).

Heiniger et al. (215) recently reported that the cytolytic activity of mouse T-cells in mixed lymphocyte cultures was inhibited if the cells were incubated in 25-OH-cholesterol; this could be reversed by adding cholesterol or mevalonic acid to the incubation media. This observation suggested that the availability of cholesterol in the culture medium and the ability of lymphocytes to synthesize cholesterol were both important factors in regulating cytotoxic functions of T-cell populations.

Peritoneal macrophages obtained from hypercholesterolemic mice showed normal tumoricidal activity (211). A high cholesterol diet did not have any detectable effect on the antitumor cell cytotoxic activity of natural killer cells from rat spleens (177), but splenic lymphocytes from the same rats exhibited a diminished in vitro proliferative response when stimulated by PHA. On the other hand, splenic cells obtained from hypercholesterolemic mice exhibited normal proliferative response when tested in vitro with either B or T-cell mitogens (211). The availability of cholesterol, either from endogenous synthesis or from an accelerated uptake from plasma of cholesterol-bound lipoprotein, was an essential prerequisite for the successful proliferation of mitogen-stimulated lympocytes (216).

5. Phagocytic activity. Tumoricidal capabilities of macrophages can be inhibited by incubating them in the presence of a high molecular weight lipoprotein, whereas incubation with a low molecular lipoprotein appeared to be stimulatory (217). The inhibition produced by the high molecular weight lipoprotein could be blocked, if cholesterol was first used to enrich the external cell membranes of the macrophage population under study.

The inhibition of phagocytosis caused by the injection of colloidal cholesterol suspensions in guinea pigs or rabbits was transient in nature and its severity was influenced by the dose of cholesterol administered (159). If the plasma membranes of peritoneal macrophages obtained from rats fed a high cholesterol diet showed an increase in their content of free cholesterol, the cells were found to have an impaired in vitro ability to phagocytize either latex particles or lipid droplets (218). Fiser et al. (207) found an increased ability of blood neutrophils from hypercholesterolemic rhesus monkeys to reduce NBT dye during a pneumococcal infection compared to the findings in similarly infected control monkeys. The influence of cholesterol availability and/or metabolism on the phagocytic capabilities of cells may be related to the requirement for a net synthesis of plasma membrane cholesterol and phospholipid after phagocytic activity (217).

Di Luzio (219) found that alimentary hyperlipemia in dogs did not alter the RES clearance of colloidal carbon or gold. However, the RES cells did function normally to remove cholesterol from plasma if excess emulsified cholesterol was injected iv. Further, if zymosan was used to induce hyperplasia and hyperfunction of the RES in rats, a subsequent acceleration of cholesterol clearance could be demonstrated. Fiser et al. (207) noted that the rate of clearance of colloidal carbon from blood was accelerated in hypercholesterolemic rhesus monkeys. Stuart et al. (175) reported that an iv injection of 10 mg of cholesterol oleate emulsion caused little change in RES clearance of colloidal carbon by mice, but larger doses caused severe inhibition of clearance and a marked increase in spleen size.

Although few available data concerning the effects of increased plasma cholesterol concentrations on the function of lymphocytes or phagocytic cells can be regarded as definitive, some role for cholesterol does seem evident. In keeping with the concept that cholesterol is necessary to allow for the synthesis of new surface membrane during periods of phagocytic activity, immunocompetent lymphocytes have been found to undergo marked changes in membrane fluidity following the microaggregation of receptor-antigen complexes. In this regard, an increased content of cholesterol in cell membranes might tend to decrease membrane fluidity and interfere with receptor movement. It was postulated that such an effect would be seen most easily during studies with relatively weak antigens (220).

6. Immunosuppression by oxidized cholesterol. Other new data (221) help reduce some of the uncertainties concerning the influence of cholesterol on immune system functions. Humphries and McConnell (221) found that oxidized cholesterol metabolites, such as 25-hydroperoxycholesterol or 25-OH-cholesterol, were immunosuppressive. This was demonstrated through studies in which mouse spleen cells were stimulated in vitro by liposomes composed of dimyristol phosphatidyl choline and cholesterol. When these liposomes contained Sepharose beads, they served as T-independent antigens which stimulated the in vitro formation of splenic plaque-forming cells. The addition of 1% or less of oxidized cholesterol molecules to such liposomes severely inhibited the formation of plaque-forming cells; this inhibition was most marked on the day of liposome administration, but diminished rapidly over the next two days (221). As few as 3×10^7 molecules of 25-OH-cholesterol proved inhibitory. This concentration equalled the very low concentration of cortisol required to inhibit splenic plaque-forming cell production following heterologous RBC immunization. Humphries and McConnell (221) postulated further that the oxidized cholesterol molecules were inhibitory on the basis of their ability to reduce 3-OH-3-methylglutaryl-CoA-reductase activity.

Further insight concerning the interaction of cholesterol with host defense and immune system functions will require data at the cellular level concerning the amounts of lipoprotein-bound cholesterol available for uptake, the rates of cholesterol synthesis from intracellular substrates, as well as the rates and quantities of cholesterol that are metabolized to oxidized products. Physicochemical details must also be uncovered concerning factors that regulate the rates and quantities of cholesterol inserted into cellular membranes. Greater information is also needed about how the presence of cholesterol influences the movement and function of the cellular membranes and their receptors, glycoproteins, immunoglobulins, and other surface markers.

VI. Minerals and trace elements

Many minerals and trace elements have biological functions which impact on host defensive mechanisms and immune system competence (see **Tables 6** and **7**). Trace elements serve as important constituents of metalloenzymes vital for maintenance of cellular viability and functions, including those that serve in host resistance. For example, iron is of key importance in oxidative functions of the respiratory enzyme chain, in glutathione reductase, and in the superoxide dismutases which participate in the intracellular killing of certain bacteria (128, 222). Divalent cations play vital roles in the maintenance of electrical potentials across cell membranes and in the function of the complement, kinin, and coagulation systems. Some metals, including chromium, zirconium, beryllium, platinum, gold, and nickel can initiate local hypersensitivity reactions (10). On the other hand, sodium, chloride, potassium, and phosphorus apparently have no unique or independent effects upon immune system function.

Iron, zinc, magnesium, and selenium have all received special attention because of their special importance in immunological system competence and host defense measures. Cobalt serves as a component of vitamin B_{12} whose immune functions were discussed earlier, but ionic cobalt may have additional effects on phagocytic cell activities. A further long list of minerals and trace elements including calcium, copper, selenium, manganese, lead, chromium, cadmium, silicon, nickel, aluminum, thorium, iodine, mercury, platinum, gallium, gold, titanium, and vanadium have been said, in one or more papers, to exert some positive or negative influence on an immune system function.

A. Iron

As a single micronutrient, iron is of major clinical importance. Vast numbers of individuals throughout the world become deficient in iron at some time in their lives. Iron deficiency accompanies most instances of PEM in children, and is of widespread and continuing importance during the menstrual years in many women. Iron deficiency is one of the most likely forms of single micronutrient deficiency to occur in the absence of any other accompanying form of malnutrition. Iron is also one of the most important micronutrients in terms of its influence on immune system functions and on other aspects of host de-

TABLE 6
Interactions of key minerals with immune system functions

	Iron excess	Iron deficiency	Zinc deficiency	Magnesium deficiency (chronic)	Selenium excess (slight)	Selenium deficiency
Host susceptibility to infection	Increased	Increased	Increased	Increased	Reduced	
Lymphoid tissues		Mitochondrial vacuolization	Marked atrophy, predominant in T-cell areas; reversible	Thymic gland hypertrophy; lymphomas may develop		
Lymphocyte counts		Reduced	Reduced *T*-cell percentages; reduced killer cell numbers			
In vitro lymphocyte transformation		Controversial; may be decreased	Consistently impaired			
Serum immunoglobulin concentration		Normal	May be reduced	May be reduced		
Antibody production		Normal or impaired	Consistently impaired	Impaired	Enhanced	Impaired
Splenic plaque cell formation			Poor		Enhanced	
Delayed dermal hypersensitivity		Controversial; may be decreased	Generally decreased			
Experimental allergic encephalitis				Inhibited		
Neutrophils:				Marked neutrophilia and eosinophilia; myeloid leukemia may develop		
a. Chemotaxis		May be increased	Impaired, reversible			
b. Phagocytosis		May be impaired	Slightly low concentrations may be stimulatory			
c. Bactericidal activity	Lactoferrin stores may become saturated	Consistently impaired				Impaired
d. Metabolic activity		Iron containing enzymes are diminished				
Complement concentrations		Normal; but C'3 may increase	Dose-dependent suppression			
Serum bactericidal activity	Impaired					
Other	Saturation of transferrin		Congenitally deficient animal models exist	Mast cell degranulation with histamine release	Immunostimulation may be potentiated by vitamin E	

TABLE 7
Toxic effects of trace and ultratrace metals on the immune system

Element	Host resistance	Antibody responses	Lymphocyte responses	Phagocyte responses	Other responses
Cadmium	Impaired	Impaired	Impaired proliferation Direct cytotoxicity Inhibits DDH	Dose-dependent stimulation or inhibition Direct cytotoxicity Macrophage spreading	
Chromium			Direct cytotoxicity Inhibited proliferation	Direct cytotoxicity	
Cobalt				Low concentrations may stimulate Macrophage spreading	
Copper	Impaired	May be impaired			Uncertain effects on local inflammatory reactions
Gold				Direct inhibition	
Lead	Impaired	May be impaired	Direct cytotoxicity Inhibited proliferation	May be stimulatory	RES inhibition
Manganese				Low concentrations may stimulate Macrophage spreading	A component of some superoxide dismutases
Mercury	Impaired	Impaired	Lymphoid tissue changes Direct cytotoxicity Inhibited proliferation		May initiate autoimmune glomerulopathy
Nickel				Macrophage spreading	
Silica				Direct cytotoxicity	
Vanadium	Impaired			Direct cytotoxicity	

fense. Either its lack or its overabundance may create problems.

The relationships between iron status and the incidence of infection in infancy have recently been reviewed (223–225). Important questions persist, however, concerning the effects of increased serum iron concentrations as a predisposing factor toward infections; the role of lactoferrin when consumed in milk and in preventing gastrointestinal infections in low birthweight infants; and the effects of iron deficiency on host susceptibility and the functional competence of the immune system.

1. Iron excess and infection. Bacteria require adequate quantities of iron to achieve their full potential for growth and replication and for the production and release of certain exotoxins. Some bacteria secrete siderophores which chelate iron in the environment to increase its availability for uptake by the bacteria. During an infectious process in animals or man, the availability of iron for acquisition by microorganisms is sharply limited by two major mechanisms. First, by the rapid sequestration of iron in tissue storage forms (6), and second, by the presence in extracellular body fluids by sizable quantities of unsaturated transferrin and lactoferrin molecules. Both of these iron-transport proteins have sufficiently strong binding affinities for iron to allow them to withhold iron from bacterial siderophores. This mechanism, under normal circumstances, serves effectively to restrict the availability of iron for uptake by invading microorganisms (224–228). Further, additional lactoferrin is released by phagocytizing white blood cells in areas of a localized inflammatory response; this process also helps to restrain bacterial growth in the immediate area. Serum trans-

ferrin, on the other hand, has more generalized effectiveness. The infection-induced abrupt redistribution of iron from serum into cellular storage sites in the liver, spleen, and marrow (6) also has a dual protective role in host defense by decreasing the availability of iron throughout body fluids and by increasing the percentage of circulating transferrin that exists in its unsaturated form.

In this frame of reference, low concentrations of serum transferrin may occur as a consequence of severe protein malnutrition. If excess therapeutic iron is given in such a situation, the lowered binding capacity of serum transferrin may become saturated. When iron-binding capacity nears saturation, iron becomes more readily available for uptake by invading microorganisms, allowing them to acquire sufficient iron to permit luxurious growth and to initiate a more virulent septic process in the host (224–233). In addition, a 1500 to 5000 MW iron chelating substance may appear in tissues of chronically iron-overloaded subjects, such as those with transfusion siderosis (234); this substance may increase the iron uptake by gram negative bacteria and promote their growth.

In 1970, McFarlane et al. (229) reported on 40 African children with severe kwashiorkor who were treated with antimalarial drugs, vitamins, folic acid, iron compounds, and a high protein diet. Many of the children died of overwhelming acute bacterial sepsis. After 2 wk of refeeding, the mean serum transferrin value of children who survived was 130 μg/dl, while those who died had a value of only 33 μg/dl. This suggested that increased availability of unbound iron in the patients with low serum transferrin concentrations may have contributed to rapidly lethal infections. Masawe et al. (230) studied African patients with iron deficiency anemias and noted that malarial attacks often developed after iron therapy was initiated. Murray and coworkers (232, 233) also reported that iron administration to iron-deficient Somali nomads with coexisting PEM was followed in about 2 wk by a marked increase in the symptomatic activation of previously inapparent malaria, brucellosis, or tuberculosis infections; a much lower incidence of infections was noted in similarly refed nomads who did not receive iron therapy (233). An abrupt increase in symptomatic malaria attacks was also noted in starved Sahelian drought patients, both children and adults, about 5 days after the start of a refeeding program that provided a grain diet to the nomads who normally consumed a milk-based diet (231, 235). These patients had very low initial serum iron-binding capacities because of their starvation-induced protein deficiency, and although the refeeding program did not include iron therapy, their serum iron values rose rapidly to saturation concentrations during the first several days of refeeding. The mechanism accounting for such an early increase in serum iron concentrations remains unexplained, but the saturation of iron-binding capacity was accompanied by increases in parasitemia from less than 5% to greater than 50%, and the onset of severe clinical malaria, often of the cerebral variety (235).

Elin and Wolff (236) conducted an instructive series of in vitro and in vivo studies on the role of serum iron concentration in infection. Since an injection of bacterial endotoxin rapidly lowers the concentration of iron in serum (6), mouse serum was collected at various times after the administration of endotoxin in an attempt to study a series of otherwise identical serum samples which contained different quantities of iron. When *C. albicans* were added to these samples, their multiplication correlated positively with the amount of iron in each serum sample (236). Other mice were then given one of four different oral doses of ferric ammonium citrate and inoculated simultaneously with bacterial endotoxin and yeast organisms. The development of low iron concentrations subsequent to endotoxin protected the mice from Candida infection, but not if endotoxin-induced hypoferremia was prevented by the administration of sufficient iron; the incidence of deaths could be related to the amount of iron in the plasma (236). Similarly, *Plasmodia berghii* infections occurred with increased virulence in Wistar rats given 10 mg of iron dextran im in comparison to findings in controls not given the iron (231). In experimental infections using virulent and avirulent strains of *S. typhimurium* in mice, lethality was greater and time-to-death was more rapid in mice injected previously with iron (237). Differences from control group findings were greatest during infections with the virulent organisms which had a greater

capability for producing siderophores and thus, presumably, a more effective mechanism to obtain iron.

Lactoferrin and transferrin are both present in human milk and typically are 56 to 89% unsaturated (224, 227). The bacteriostatic effect of human milk on *E. coli* is abolished, however, if iron is added to the milk in concentrations sufficient to saturate these proteins. The feeding of milk with unsaturated lactoferrin was found to lower the number of *E. coli*, strain 0111, in the intestine of guinea pigs, whereas iron-saturated milk did not (228).

Neutrophils are known to produce lactoferrin. When rabbit neutrophils were allowed to phagocytize a ferritin-antibody complex, their intracellular lactoferrin stores became saturated with iron, and the "iron-fed" neutrophils subsequently lost their bactericidal capacity (228). As a control for this study, apoferritin-antibody complexes which did not contain iron were also "fed" in comparable amounts to control neutrophils, but phagocytosis of noniron portions of the complex caused no reduction in the neutrophilic capacity for killing test bacteria (224).

In contrast to these studies, Hill (238) prevented the hypoferremia and anemia which typically accompany a *S. gallinarum* infection of chicks either by the use of dietary feedings of iron or by the injection of iron-EDTA. The iron-treated chicks showed fewer bacteria in their blood, liver, and spleen than a control group. In contrast, iron administration did not protect chicks during *E. coli* infections. Thus, the effects of hyperferremia may vary with the species of invading microorganism.

A general interpretation of the mechanisms which account for increased virulence of certain infections, or the symptomatic emergence of subclinical malaria, brucella, or tuberculosis infections (233) can be based upon the common variable in both the animal and human studies, i.e., the degree of saturation of iron-binding proteins in plasma. This has become a widely supported hypothesis and can explain the increases in incidence rates and virulence of certain infections.

However, in focusing primarily upon organism virulence, this hypothesis ignores the important host-centered roles of iron in generalized resistance, immune system competence, and the phagocytic and microbicidal capabilities of body cells. These iron-related aspects of host defense and immune function help explain many of the clinical and experimental observations. Repletion of a starved individual with energy substrates, amino acids, and many other individual essential micronutrients (including iron) often allows a previously malnourished patient to develop a fever, produce new white blood cells, generate an inflammatory or granulomatous response, and to initiate a wide array of integrated immunological responses. The reappearance of these defensive capabilities in a newly re-fed patient can be used to explain the reemergence of a capacity to generate fever, inflammatory reactions and/or granulomatous lesions without a need to postulate an increase in the virulence of an infectious microorganism. High fever or severe inflammatory or granulomatous reactions can be deleterious in a recently starved patient. Their potential dangers point out the clinical importance of nutrient interactions with host defense mechanisms in a starved patient. We need to understand the basic mechanisms at play in such interactions to devise the safest possible methods for correcting severe malnutrition.

2. Iron deficiency and infection. Despite its obvious difference from a state of iron excess, a deficiency in body iron can also increase host susceptibility in infectious diseases. Under such circumstances, an iron-deficient subject becomes more susceptible to infectious microorganisms primarily because of an iron-related impairment in the functional capabilities of immune mechanisms and other host defenses important for maintaining resistance.

Iron-deficient rats have an increased susceptibility to intestinal infections with *S. typhimurium* (239–241), possibly because the deficient rats are unable to produce and deliver sufficient numbers of myeloperoxidase-containing phagocytic cells to the gut to eliminate the enteric pathogens. Iron availability may also influence microbial colonization on body surface membranes (242).

Immune functions have been studied in both patients and experimental animals with varying degrees of iron-deficiency anemia. While most publications indicate that iron deficiency produces a wide range of adverse consequences in terms of immune functions

and phagocytic activities, not all investigators have reached identical conclusions about abnormalities in specific immune functions. Many of the reported clinical studies were conducted in patients with coexisting PEM and other micronutrient deficits in addition to the deficiency of iron. Many studies were conducted in subjects with parasitic infestations or in those with overt or subclinical infections, and some were conducted during the early convalescent phases of infection. In many human and animal studies, the presence or absence of an infectious process was neither mentioned nor considered when interpreting the data. In such instances, changes in immune system functions actually due to a coexisting infectious process may have erroneously been ascribed to one of the nutritional variables under study.

3. Lymphocyte numbers and tissue effects. Relatively little information is available concerning possible changs in lymphocyte subpopulation numbers during iron deficiency anemia in man. However, Fletcher et al. (242) reported that total lymphocyte numbers were reduced, and Jarvis and Jacobs (243) found that 90% of lymphocytes from iron-deficient patients had vacuolar changes in their mitochondria when examined by electron microscopy. A decreased percentage of T-lymphocytes in the peripheral blood of iron-deficient children was corrected by iron therapy within a 4-wk period (118, 126, 244). Iron-deficient rat pups show lymphoid tissue abnormalities (245) with impaired lymphopoiesis of both B- and T-cells. Iron-deficient mice also have depressed peripheral lymphocyte counts and defective cells in their spleens (246).

4. Humoral immunity. No clinical abnormalities have been reported in serum immunoglobulin concentrations (247–249) or in salivary IgA values (248) in association with iron deficiency. Normal values for total serum complement have been reported by Chandra et al. (247) and Macdougall et al. (248) but the latter group observed increased C'3 values in children with iron deficiency. The antibody responses to immunization with tetanus toxoid and typhoid vaccines were normal in children with iron deficiencies, even though some patients had concurrent infections (247).

Nalder et al. (250) studied the effects of iron deficiency on antibody synthesis of rats by decreasing dietary iron in 10% increments. A decrease in antibody production after immunization with tetanus toxoid was proportional to the decline in iron intake. This decrease in rat antibody titers appeared to be a more sensitive indicator of dietary iron intake than measurements of Hb, serum or liver iron, or body weight.

5. Cell-mediated immunity. The in vitro responsiveness of peripheral blood lymphocytes from iron-deficient patients to various mitogens or antigens has been the topic of some controversy. Bhaskaram and coworkers (118, 244) reported decreased responsiveness of peripheral lymphocytes to PHA in iron-deficient patients, which was improved by iron therapy in the absence of any other nutritional manipulation. Fletcher et al. (242) also reported diminished in vitro transformation of lymphocytes by PHA. Jacobs and Joynson (251) reported diminished in vitro lymphocyte response to tuberculin and Candida antigens but noted a normal lymphocyte response to PHA. Joynson et al. (252) also reported diminished transformation of blood lymphocytes with PPD and Candida antigens. Using PHA or Candida antigen stimulation, Macdougall et al. (248) reported a diminished [^{3}H]thymidine uptake by peripheral lymphocytes from 20 children with iron-deficiency anemia and seven with latent iron deficiency. In contrast to normal values in controls, lymphocytes from 26 patients who were free of infection, but with iron deficiency, showed diminished in vitro responsiveness to phytomitogens, whether or not the patients were anemic (249). Srikantia et al. (253) observed a depressed [^{3}H]thymidine uptake response to PHA in peripheral blood lymphocytes of 88 noninfected children with hemoglobin values less than 10 g/dl.

In contrast, impaired lymphocyte responsiveness to PHA was not found by Gross et al. (49) in patients with iron-deficiency anemia. Similarly, Kulapongs et al. (254) studied eight Thai children with severe iron-deficiency anemia, seven of whom had a hookworm infestation, and found normal blastogenic transformation and [^{3}H]thymidine uptake values by blood lymphocytes stimulated by PHA. Suskind et al. (255) performed studies in noninfected Thai children with iron-deficiency anemia and mean hemoglobin values of 3.5 g/dl; after therapy with a dextran-

iron complex, hemoglobin increased to 11.4 g/dl, but the values for in vitro lymphocyte transformation responses to PHA measured both before and after therapy were not different from those of healthy control children.

Buckley (256) and Suskind and Adeniyi-Jones (257) have discussed these contradictory data and offered possible explanations for the observed differences on the basis of a large number of important nonidentical variables, including generalized nutritional status, infection, comparability of controls, timing of the studies, methods used in lymphocyte culture studies, folate nutriture, severity of anemia, and timing of iron therapy.

An impaired DDH response to skin test antigens has been reported (118, 247, 251) in children with iron deficiencies, some of whom showed anemia and/or concurrent infectious diseases. Strauss (258) suggested that the inflammatory response required for a positive dermal skin test result was diminished by iron deficiency. In contrast, Gross et al. (49) were unable to find skin test abnormalities in patients with iron-deficiency anemia. However, when iron-deficient mice were purposefully sensitized with dinitrofluorobenzene (259), the inflammatory reaction was minimal and in vivo incorporation of uridine in lymphocytes was impaired.

Deficits in other indicators of CMI may occur in iron deficiency. Killer T-cell activity was reduced in iron-deficient mice (260). A diminished production of macrophage-inhibition factors by lymphocytes of patients with iron-deficiency anemia has been reported by Jacobs and Joynson (251) and by Joynson et al. (252). Rodday et al. (261) found that a combination of irradiation and iron deficiency in rats did not affect their ability to reject 20 to 50 $\times$ 10^6 allogeneic marrow cells. In contrast, iron deficiency reversed the usual 5-day delay observed before initiating erythropoiesis in spleens of lethally irradiated Lewis rats grafted with 4 to 35 $\times$ 10^6 syngeneic marrow cells. Unlike the findings with many other micronutrients considered in this review, surprisingly little work has been done in iron-deficient experimental animals with regard to alterations in CMI. Such studies should lend themselves to purposeful experiments with well-planned design features in order to explore cell-mediated responsiveness in appropriately selected iron-deficient animal species and inbred strains. Careful controls are needed to eliminate important variables such as intercurrent infections and coexisting nutrient deficiencies. Immunological phenomena under consideration must also be studied with different degrees and durations of iron deficiency as well.

6. *Phagocytosis.* Another important aspect of iron deficiency is its effect on the functional activity of phagocytic cells (262). In this regard, iron deficiency can interfere with either nucleic acid synthesis and/or with other cellular mechanisms which require iron-containing metalloenzymes. Hershko et al. (263) reported that bone marrow cells of iron-deficient patients showed a diminished content of nucleic acids and a slow rate of [^{3}H]thymidine incorporation into DNA. These defects were corrected by iron therapy. Hoffbrand et al. (264) ascribed nucleic acid defects to an iron-containing ribonucleotide reductase. Deficient activity of this enzyme led to an intracellular increase in deoxythymidine triphosphate and a concomitant reduction of deoxyadenosine triphosphate. In addition, a diminished availability of iron for the myeloperoxidase enzymes of phagocytic cells may impair the ability of these cells to kill ingested bacteria and fungi.

Beard and Weintraub (265) reported that patients with iron-deficiency anemia, but without coexisting abnormalities of vitamin B_{12} or folate, showed hypersegmentation of neutrophil nuclei and an occasional metamyelocyte in the circulating blood. These abnormalities were reversed with iron therapy. Chandra (266) reported that the neutrophils of children with iron-deficiency anemia showed reduced NBT dye-reducing values but normal phagocytic abilities. In contrast, Macdougall et al. (248) reported that children with iron deficiencies, with or without anemia, had circulating neutrophils with normal NBT dye test values but with increased chemotactic activity.

The major functional abnormality of iron-deficient phagocytic cells is a primary defect in bacterial killing ability. Defective bactericidal functions have been reported by several groups (247, 248, 262, 266). In contrast, Suskind et al. (255) found no defect in the bactericidal activity against *E. coli* by neutrophils from severely anemic children who were free of infections, and Kulapongs et al. (254)

found abnormal phagocytic and bactericidal activity in the neutrophils from only one of eight Thai children with severe iron-deficiency anemia and coexisting hookworm infection. Arbeter et al. (262) emphasized the role of iron deficiency rather than that of protein or energy in causing bactericidal dysfunction in adults and children with severe malnutrition; they postulated that the lack of iron was most crucial for the activity of myeloperoxidase enzymes in phagocytic cells.

B. Zinc

As with iron, zinc deficiency can impair a variety of immune functions and host defensive mechanisms. Unlike iron, however, most studies of zinc deficiency have been performed in laboratory animals rather than man. Since there are no major body storage depots for zinc, severe deficiency is produced easily and quickly. This convenience has led to studies of zinc deficiency in relation to many aspects of immune function not previously evaluated with other micronutrients.

Immunological studies have been conducted in some individual patients whose zinc deficiency developed as a secondary consequence of severe medical or surgical illness. In such patients, zinc deficiency usually appeared in combination with other nutritional deficiencies.

Zinc deficiency is a common feature of an inherited disease, acrodermatitis enteropathica (267–272). A diminished ability of intestinal cells to absorb zinc appears to constitute a primary metabolic defect in this disease, with multiple immunodeficiencies and increased susceptibility to infection occurring as secondary manifestations of the diminished content of zinc within body cells. Zinc deficiency in acrodermatitis enteropathica leads to an increased susceptibility to infection (273–276). A large daily intake of oral zinc reverses these infections as well as the immunodeficiencies and allows the skin lesions to heal. A somewhat analogous congenital defect of zinc absorption has been identified in one variety of Friesian cattle; this disease proves rapidly fatal unless treated by large dietary supplements of zinc (277).

1. Lymphoid tissue effects. Zinc deficiency has a profound impact upon the anatomy of lymphoid tissues in experimental animals (29, 275–288). Depending upon its severity and duration, zinc deficiency produces hypoplasia of the thymus, spleen, lymph nodes, Peyer's patches, and other intestinal lymphoid tissues; these changes can progress to virtual atrophy. Thymic involution involves primarily the cortical areas (279). Both T- and B-lymphocyte populations are affected by zinc depression, but cell depletion from lymph nodes involves primarily the T-dependent areas (288). Despite the severity of hypoplastic lymphoid changes, restoration of zinc to an experimental animal is generally followed by the correction of anatomical defects within a period of several weeks. Even in the presence of marked lymphoid structure hypoplasia in zinc-deficient BALB/c mice, Frost et al. (29) noted increased rates of circulation of lymphocytes through the spleen and peripheral and mesenteric lymph nodes.

In a single study in patients, Golden et al. (273) used chest x-rays to demonstrate thymic atrophy in eight children with coexisting PEM and zinc deficiency. After therapy with high caloric, high protein diets, the thymus glands remained small and plasma zinc remained low. The subsequent addition of 2 mg/kg/day of zinc acetate to the diet significantly increased both plasma zinc values and thymus size within a 10-day period.

2. Lymphocyte numbers. Zinc deficiency leads to altered lymphocyte counts in experimental animals. DePasquale-Jardieu and Fraker (284) attributed some of the depression in T-helper lymphocyte functions to an increase in plasma corticosterone concentration from 40 to 115 μg/dl in zinc-deficient mice. However, approximately half of the decrease in T-lymphocyte numbers occurred before the increase occurred in steroid values. A subsequent study (285) strengthened the concept that zinc-requiring effects are independent of glucocorticoid requirement. Fernandes et al. (275) showed that zinc deficiency in several strains of mice led to depressed natural killer cell activities, diminished T-lymphocyte killer activities against inoculated allogeneic tumor cells, and a depression in both relative and absolute numbers of Thy-1.2 positive cells, and in contrast, to an increase in lymphocytes bearing Fc receptors. These alterations in lymphocyte subpopulations did not occur in pair-fed or normally fed control groups. Chandra and Au (286) also noted depressed base-line activ-

ity and antibody-dependent cytotoxic activity of killer lymphocytes from zinc-deficient rats.

3. Humoral immunity. A large number of studies have recorded diminished appearance of splenic plaque-forming cells in response to the inoculation of experimental animals with sheep RBC (29, 275, 278–280, 296–298, 301) or tumor cells (286, 288) as the stimulating antigens. The lymphocyte requirement for zinc can be shown in rat pups fed by zinc-deficient lactating dams (289). The lymphocytic requirement for zinc appears somewhat less in females (290) and is less than the requirement for sustained growth in rats (291). A brief rebound to greater-than-normal responsiveness may follow zinc repletion (290).

The wide use of splenic plaque-forming cell counts as an index of humoral immune stimulation against RBC in many studies in zinc-deficient animals has not generally been accompanied by concomitant measurements of antibody titer responses. Based on the diminished formation of plaque-forming spleen cells, however, antibody titer responses would be expected to be marginal at best. Brummerstedt et al. (277) did record abnormally low antibody responses in zinc-deficient Friesian cattle to immunization with tetanus toxoid; normal responsiveness was restored by zinc therapy.

Little has been reported concerning basal serum Ig concentrations in zinc-deficiency states. Cunningham-Rundles et al. (292) described a severe depression of serum IgG in three patients with zinc deficiency and generalized PEM. In contrast to values in normal and pair-fed controls, groups of mice with varying degrees of zinc deficiency showed a highly disordered profile of serum Ig distribution, with depressed values after sheep RBC of serum IgM, IgG_{2a}, and IgA, but a marked increase in IgG_1 (287).

4. Cell-mediated immunity. In accord with the abnormal function of lymphocyte subpopulations in zinc-deficient animals suggested by the poor production of splenic plaque-forming cells after allogeneic RBC immunization, there is a marked deficiency in lymphocyte responses to in vitro mitogens or antigens (269, 274, 292–297). Lymphocytes from zinc-deficient patients have also shown diminished proliferative responses to mitogens and ubiquitous antigens on in vitro testing; these abnormalities were corrected by zinc therapy (292). Gross et al. (293) noted diminished in vitro responses to T-cell (PHA and Con A) and combined T- and B-cell (pokeweed) mitogens in rat lymphocytes obtained from spleen, thymus, and peripheral blood; normal responses were present in lymphocytes from pair-fed controls. The in vitro addition of levamisole, an immunopotentiating drug with a yet uncertain mode of action, restored normal responsiveness of zinc-deficient lymphocytes to PHA stimulation (293). This drug, however, had no discernible effect on lymphocytes from control rats.

Depressed lymphocyte responses to mitogens was also observed in three children who developed acrodermatitis enteropathica-like skin lesions while receiving a total iv alimentation regime that inadvertently permitted the development of zinc deficiency; both the immunological defects and the skin lesions were reversed by the oral administration of zinc. An in vitro deficiency in lymphocyte mitogen response was restored to normal by zinc feedings in another patient who developed an isolated zinc deficiency while on iv alimentation (297). In a single zinc-deficient patient without coexisting PEM, blood lymphocytes showed increased in vitro responses to stimulation with PHA, normal responses to pokeweed mitogen, but diminished responses to Candida antigen (274).

A very narrow in vitro concentration range of zinc chloride, approximating *10 μM*, was shown by Hart (295) to be optimal in hamsters for mitogenic response of lymph node cells but not for thymus or spleen cells. The optimal concentration of zinc was increased for mitogenic responses to LPS but not Con A. Similar optimal response ranges were found for guinea pig lymph node and spleen cells but at a much higher, i.e., 50 μM, concentration of zinc chloride.

5. Skin test responses. Impaired DDH responses were also noted when testing zinc-deficient Friesian cattle for sensitivity to previously administered BCG or DNCB (277). When viable BCG were used to immunize zinc-deficient guinea pigs (298), the bacilli showed an enhanced growth, but the development of DDH was impaired. Similarly, a single patient with isolated zinc deficiency failed to develop positive tests when sensitized with DNCB (297). However, the most

impressive study of impaired DDH responsiveness was accomplished in zinc-deficient children by Golden et al. (299), who administered ubiquitous antigens in an identical series of skin tests on both arms of each child. One arm was then treated with a local application of zinc sulfate in an emulsified ointment, whereas, the other arm, which served as a control, was treated by the same ointment but without its content of 1% zinc sulfate. The subsequent DDH reactions were significantly larger in the arms treated with topical zinc in children with moderate zinc deficiency implying that local absorption of zinc from the skin was able to restore sufficient local cellular activity to permit a full DDH response. Children with severe zinc deficiency, however, failed to develop positive skin tests on either arm (299).

6. Phagocytic cell functions. A number of studies suggest that the intracellular concentration of zinc helps to determine the magnitude of oxygen consumption, phagocytic activity, and bactericidal capacities of neutrophils (300).

Lennard et al. (301) studied the neutrophils of patients with burns that covered 30% or more of body surfaces; a critical in vitro concentration range of zinc was found to increase the phagocytic capacity of the cells. However, zinc concentrations did not correlate with NBT test responses. Westen et al. (272) also noted impaired chemotaxis of monocytes and neutrophils from zinc-deficient patients with acrodermatitis enteropathica; this was corrected either by giving zinc therapy to the patient, or by adding zinc sulfate in vitro during incubation and testing of the cells. Neutrophils from hemodialysis patients with a subclinical zinc deficiency showed diminished chemotaxis (302) although their lymphocytic functions were still intact; the neutrophilic dysfunction was corrected by zinc supplementation. Patrick et al. (303) noted that dietary zinc supplements given to children recovering from severe PEM appeared to stimulate sodium transport by their peripheral blood leukocytes. The most definitive studies of phagocytic cell function have been reported by Chvapil et al. (304–306). Both in vitro and in vivo studies demonstrated important functional effects of zinc on cell membrane and activities related to phagocytosis. Increased quantities of zinc inhibited the membrane fluidity of macrophages, neutrophils, mast cells, and platelets. This was associated with inhibition of platelet aggregation, inhibition of histamine release by mast cells, and diminished consumption of oxygen in neutrophils along with diminished phagocytic and bactericidal activities. In contrast to the diminished neutrophilic activities noted with concentrations of zinc ion somewhat higher than those found in normal plasma, concentrations somewhat lower than normal tended to stimulate in vitro oxygen consumption as well as both phagocytic and bactericidal activities. Zukoski et al. (307) have also reported inhibition in both the migratory and phagocytic activities of peritoneal macrophages obtained from rats and guinea pigs that have been fed for 3 days on a high (2000 ppm) zinc diet. These macrophages showed a smooth rounded surface by electron microscopy rather than their usual ruffled appearance, and they exhibited diminished activities of ATPase and NADPH oxidase enzymes.

7. Other effects. Montgomery et al. (308) recently studied the classical complement pathway in guinea pig serum over a wide range of zinc concentrations. At in vitro concentrations greater than 200 μM, all components were strongly inhibited. At 25 μM, C2, C3, and C6 showed moderate inhibition but C1, C4, and C9 values remained normal. C5 was enhanced at zinc concentrations of 25, 50, and 100 μM. None of the zinc-induced changes was permanent and all occurred prior to complement activation and/or binding to cell surfaces.

The thymic atrophy observed in zinc-deficient animals and man is accompanied by a deficient thymic gland output of its measurable humoral factors (269). Cunningham-Rundels et al. (292) noted a lowering in serum thymopoietin values in three zinc-deficient patients; normal values were restored following treatment with zinc. Iwata et al. (280) reported a marked decrease in serum thymic factor activity in A/JAX mice fed a zinc-deficient diet in comparison to values measured in either pair-fed or normally fed control mice.

C. Magnesium

Magnesium ions are required for participation of properdin in the alternate comple-

ment pathway. However, deficits in magnesium have not been reported to cause changes in the immune system or blood leukocyte numbers of human subjects. In contrast, marked changes occur if mice or rats are fed for extended periods of time with diets moderately deficient in magnesium.

1. Leukocytosis. Rats fed a magnesium-deficient diet developed persistent leukocytosis; this included increased numbers of neutrophils, mononuclear cells, and especially eosinophils which increased as much as 1000% (309–314). The extremely high eosinophil numbers were present in blood and peritoneal fluids of both normal and adrenalectomized rats; massive increases in tissue eosinophil numbers were also found in the lungs, submaxillary glands, and lymph nodes (310). Magnesium-deficient rats (311, 312) developed dermal hyperemia in addition to leukocytosis and eosinophilia.

2. Mast cell changes. Magnesium-deficient rats exhibited a deficiency of mast cells in the peritoneum; but at the same time, mast cells appeared in liver sinusoids of magnesium-deficient, adrenalectomized rats (310). McCreary et al. (311, 312) observed extensive degranulation of rat mast cells, increased excretion of histamine in the urine, and a progression of the persistent leukocytosis to leukemic changes. Bois (314) also found an abnormal distribution and diminished granularity of mast cells but was unable to detect a consistent alteration in urinary histamine excretion of rat studied after 10, 20, or 60 days of magnesium deficiency. The histamine content of mast cells declines within 10 days of magnesium deficiency and thereafter remains at, or below, one-third of normal values (315).

3. Leukemia/lymphoma. Battifora et al. (313) confirmed the occurrence of myelogenous leukemia in rats if sustained leukocytosis was not corrected by reversing the magnesium deficit. The leukemia could be transplanted to newborn rats. In a series of additional studies concerning the susceptibility of magnesium-deficient rats to malignant changes, Hass et al. (316, 317) observed that 20% of Sprague-Dawley rats developed a malignant lymphoma within 8 to 24 weeks after initiating a low magnesium diet (3 to 5 mg/100 g of feed). An additional 5% of the rats on this prolonged low magnesium diet developed myeloid leukemia between 28 to 60 wk. Both diseases could be transmitted by inoculation of the live cells. After about 8 wk on such a magensium-deficient diet, preestablished immunity to live lymphoma cells was lost, and rats developed tumors after receiving only small "immunizing" doses of lymphoma cells (316, 317). McCreary et al. (318) also noted spontaneous appearance of malignant lymphomas in magnesium-deficient rats and found that such rats were more susceptible to the transmission of lymphomas from injected cells.

4. Immune responses. Depressed serum IgG values, noted in magnesium-deficient rats (319) were reversed within 24 h after initiation of magnesium repletion; a marked rebound to higher serum IgG values then occurred over a 2-wk period. Elin (320) found depressed concentrations of IgG_1, IgG_2, and IgA in magnesium-deficient mice, but others (317, 321) have reported normal serum IgG concentrations in magnesium-deficient rats.

On the other hand, investigators are in agreement that immunizations of magnesium-deficient rats or mice are not followed by normal humoral immune responses. Deficient mice showed only a minimal appearance of splenic plaque-forming cells in response to immunization with sheep RBC (320). Sheep RBC immunization did not stimulate the normal formation of hemolysins or HA antibodies in deficient rats (317, 321). In addition, magnesium-deficient rats exhibited a diminished ability to be immunized by live lymphoma cells (317) or to develop experimental allergic encephalomyelitis after inoculation with brain tissue antigens (311, 317).

In contrast to deficiencies of many other individual micronutrients which lead typically to thymic atrophy, magnesium-deficient mice have marked thymic hyperplasia. Despite this hyperplasia, magnesium-deficient rats tend to lose their cell-mediated immunocompetence and become more susceptible to encephalitozoon infections (317).

D. Selenium

Although ^{75}Se is taken up in vitro by human lymphocytes by a sulfhydryl-requiring mechanism (322), no reported studies describe any effects of selenium on immune functions of man. However, Mulhern et al. (323) found that the offspring of selenium-

deficient mice had an impaired splenic lymphocyte response to sheep RBCs (323). Spallholz and colleagues (324–326) evaluated the effects of increased or reduced quantities of selenium in the diet of mice on the adequacy of a primary immune response to sheep RBC. Either a deficiency of dietary selenium or the administration of toxic doses served to reduce HA antibody titer development. In contrast, diets containing 1.25 to 2.25 ppm selenium led to an increase in the primary immune response to sheep RBC as measured by the increased development of splenic plaque-forming cells as well as by higher IgM and IgG titers of HA antibodies. Similarly, an ip injection of 5 μg selenium given either before or concomitantly with the sheep RBC inoculation also led to increased HA titer responses in comparison to those of normal controls.

Desowitz and Barnwell (327) found that the addition of 2.5 μg/ml of selenium to the drinking water of mice potentiated the effects of an experimental schizont-merozoite *P. berghei* vaccine, whether or not an adjuvant was used. Upon subsequent challenge, vaccinated mice receiving the added selenium had lower malarial counts and shorter durations of parasitemia in comparison with those of immunized control mice which did not receive the selenium supplement.

Sheffy and Shultz (328) also showed that selenium was immunostimulative if given to dogs in a modest excess. On the other hand, selenium deficiencies were found to suppress the immune responses of dogs. The suppression in selenium-depleted dogs was greatest when they were fed diets high in PUFA. Conversely, the immune deficiencies could be corrected with vitamin E supplements. Noguchi et al. (139) also showed that vitamin E and selenium interacted with respect to their influence on immune system functions.

Selenium deficiency in rats and cattle impaired the bactericidal activity of neutrophils against yeast, although it did not alter phagocytic function (329). Defective neutrophils exhibited low glutathione peroxidase activities.

E. Calcium

Calcium is receiving new attention as a regulator of lymphocyte function. In addition to its roles in influencing cellular excitability and the activation of complement and coagulation system components, important new cellular effects of ionic calcium are being recognized.

1. Cellular uptake. When human blood lymphocytes are stimulated in vitro by PHA, they normally accumulate calcium ions from the surrounding medium in amounts which can be quantitated by the use of ^{45}Ca (330). Lipoproteins that inhibit PHA-stimulated lymphocyte proliferations diminish the uptake of radiolabeled calcium by the stimulated cells. The inhibition of ^{45}Ca uptake is greatest with an intermediate density lipoprotein, evident with a low density lipoprotein, and least marked with a very low density lipoprotein (330). This progression correlates directly with the ability of these various lipoproteins to inhibit PHA-stimulated lymphocyte proliferation. In order to be inhibitory, these lipoproteins must first bind to specific preexisting receptors on the lymphocyte surfaces by an action which can be reversed by heparin. The late addition of a low density lipoprotein inhibitor to cultures stimulated 5 h earlier by PHA causes the lymphocytes to release all ^{45}Ca taken up during the preceding 5-h period.

Mobilization of intracellular calcium ions appears to be an absolute requirement for oxygen radical generation and degranulation in activated human neutrophils (331).

2. Calmodulin. A recently recognized cellular protein, calmodulin, is now known to modulate the role of calcium in terms of its intracellular actions, including those affecting the synthesis and function of the prostaglandins (332, 333). Calmodulin is a heat-stable, relatively acidic small protein with an unusually high content of aspartic and glutamic acids. These acids appear to contribute to the ability of each molecule to bind four calcium ions. Binding of calcium causes an individual binding site to assume a helical coil configuration which, in turn, may allow the calcium-calmodulin complex to interact with receptor sites on enzymes and cellular membranes. Regulatory effects of calcium on lymphocytic and phagocytic cell functions will undoubtedly prove to involve an interaction with calmodulin; these exciting new findings concerning the actions of calcium are opening new areas for the molecular studies of immune function.

F. Cadmium

Cadmium has been studied in vivo with respect to its effects on generalized host resistance and responsiveness to immunization, and in vitro for its direct effects on neutrophil, macrophage, and lymphocyte functions.

Inclusion of subtoxic quantities of cadmium sulfate in the drinking water of mice increased their susceptibility to encephalomyocarditis virus infection (334) while an iv injection of 0.6 mg/100 g increased the lethality of endotoxin in rats (335).

Hill (238, 336) noted that a modest nontoxic increase in dietary cadmium intake increased the resistance of chickens against an experimental infection with *S. gallinarum* compared to infections in control groups. On the other hand, toxic loads of 100 ppm dietary cadmium diminished the resistance of chickens to the same bacterium. The diminished resistance disappeared, however, soon after the dietary excess was discontinued.

1. Immune responses. After accumulating a massive 4.55-g excess of body cadmium because of the addition of cadmium chloride to drinking water for a 70-day period (337), rabbits demonstrated a diminished formation of neutralizing antibody after three weekly inoculations with pseudorabies vaccine. In a series of studies Koller and collaborators (337–340) showed that prolonged feeding of subtoxic doses of cadmium to mice reduced the number of rosette-forming B-cells in the spleen and marrow, and also reduced the splenic lymphocyte formation of IgM and IgG after primary and booster inoculations with sheep RBC. These immunosuppressive effects persisted for months after feedings were discontinued. Prolonged feeding of cadmium also led to inhibited mitogenic responsiveness of splenic lymphocytes (341). Single doses of cadmium also caused changes in antibody production, but these were influenced by the route of administration (339). Effects of single dose administration were also influenced by their timing in relation to that of antigen administration (342). Gallagher et al. (343) reported that 10^{-7} to 10^{-3} *M* concentrations of cadmium caused cell death of in vitro mouse lymphocyte cultures. Lower concentrations led initially to inhibition of the proliferative response to LPS, and cellular RNA synthesis, but to an increase of cellular hexokinase activities.

a. Delayed Hypersensitivity. Chronic oral administration of cadmium to mice led to an inhibition of cell-mediated immunity to sheep RBC inoculations as assessed by local footpad swelling (344).

2. Phagocytic cell effects. Chvapil et al. (305) found that 50 μM concentrations of cadmium were without effect on either the phagocytic activity or the respiratory burst of dog granulocytes studied in vitro. Chronic oral feedings of subtoxic doses may stimulate phagocytic activity in macrophages (345). On the other hand, Loose et al. (346) reported that the phagocytic activity of neutrophils, peritoneal macrophages, and alveolar macrophages obtained from BALB/c mice were inhibited when the cells were inoculated with 8×10^{-3} to 8×10^{-1} mEq/L of cadmium salts, and that the respiratory burst which accompanied phagocytosis was depressed (347). Cadmium also reduced the bactericidal activity of alveolar macrophages but not that of the other cell types studied. Macrophages are killed in vitro by 0.1 m*M* ionic cadmium (348). They take up cadmium, possibly through a cell wall mediated mechanism (349) and store it in association with a metallothionein-like protein (350). Rabinovitch and Destefano (351) studied the spreading of macrophages on glass surfaces coated with antigen-antibody complexes. The presence of divalent cations, including manganese, cobalt, nickel, and zinc caused the macrophages to "spread." Cadmiun ion effects were smaller, while trivalent aluminum or quadrivalent thorium ions were without effect. The "spread" macrophages showed normal phagocytic activity.

G. Lead

Excess lead causes immunological changes comparable to those caused by an excess of cadmium. Subtoxic oral doses reduce host resistance to bacterial (334, 335) and viral (334) infections.

1. Humoral immunity. Hoffman et al. (354) investigated the possible toxic effects of lead on humoral antibody formation by feeding lead acetate solutions to lambs, 5 days/wk for a 12-wk period, in doses of 0, 2, 4, 8, and 16 mg/kg; these doses produced final mean blood lead concentrations of 12, 37, 57, 68, and 99 μg/dl, respectively. Despite these values, no effects were noted on basal serum

concentrations of IgM or IgG, and no differences were seen in the development of HA titer responses to immunization with heat-killed *Serratia marcescens*. In contrast, rabbits given lead acetate in the drinking water for 70 days developed an accumulated total body dose of 31.61 g and showed depressed neutralizing antibody responses after a 3-dose series of pseudorabies virus immunizations (337). Mice given lead acetate in their drinking water in amounts of 0, 13.75, 137.5, or 1375 ppm for 56 days showed a dose-related decrease in the formation of splenic plaque-forming cells after both primary and secondary immunizations with sheep RBC (355). Others have confirmed the abnormal responses to sheep RBC immunizations (345, 356); B-cell rosette formation is reduced in marrow preparations (340).

2. Splenic lymphocyte responsiveness. Neilan and Taddeini (357) added 30 ppm of lead to the drinking water of mice for a total of 15 wk; no important differences from control values were noted in T- and B-lymphocyte percentages in peripheral blood or in the in vivo stimulation of lymphocytes in the presence of ConA, LPS, or Poly(I)·Poly(C); however, stimulation by PHA was inhibited by approximately 40% in comparison to controls. Gaworski and Sharma (341) also reported an inhibition of PHA-induced mitogenicity.

Gallagher et al. (343) added ionic lead to mouse lymphocyte cultures in concentrations ranging from 10^{-3} to 10^{-7} *M*. Higher concentrations led to cell death and lower concentrations inhibited lymphocytic proliferative responses induced by LPS. However, the lower concentrations of lead appeared also to increase cellular RNA synthesis and the activity of cellular hexokinase.

3. Phagocytic cell effects. Injections of lead acetate to mice stimulated a neutrophilic leukocytosis (358), while the addition of lead to drinking water stimulated phagocytic activity of peritoneal macrophages (345). In contrast, the RES clearance of colloidal carbon was inhibited in rats (359).

H. Copper

Experimentally induced deficits or excesses of copper have each been reported to increase the severity of infections in laboratory animals. Copper deficiency has increased the severity of salmonella infections in rats (360) and trypanosomiasis in mice (361), while excesses have increased the severity of salmonellosis in chickens (336) and yeast infections of mice (362). Infection is a frequent cause of death in patients with Menkes kinky-hair syndrome, and inherited disease with defective copper metabolism and low serum copper concentrations (363). The results of extensive immunological studies in one infant with this syndrome were generally normal, although a poor conversion of IgM to IgG was found following booster immunization with a test antigen (363). Mice fed a diet deficient in copper also showed a reduced antibody response of splenic lymphocytes after sheep RBC inoculations (364).

No attempt will be made to review the question as to whether externally applied metallic copper, copper salts, or copper in combination with a chelating agent such as D-penicillanine, have a beneficial effect on localized inflammatory reactions. In one study, however, an induced dietary deficiency of copper did increase the severity of an experimentally induced inflammatory lesions in rats (365). Copper-deficient rats have exhibited a deficient reticuloendothelial system response during induced salmonellosis (9). A 50-μM concentration of ionic copper in vitro was optimal for phagocytic activity and oxygen uptake of dog granulocytes (306). Some superoxide dismutases of phagocytic cells contain copper and zinc, others manganese, and still others iron at the active site. These enzymes have a key role in bactericidal activities of these cells, and constitute possible sites of trace element influence.

An chronic excess of copper induced in sheep has led to the development of direct Coombs test positive red blood cells (366).

I. Cobalt

A 50-μM concentration of cobalt ions was inactive with respect to altering phagocytic activity of dog granulocytes studied in vitro (306). Divalent cobalt, however, was effective in inducing macrophages to spread on a glass surface layered with antigen-antibody complexes (351). Stossel (367) reported that human neutrophils and rabbit alveolar macrophages increased their phagocytic uptake of albumin-coated paraffin oil particles when studied in the presence of ionic cobalt.

J. Manganese

Manganese resembles cobalt in being able to stimulate an equal degree of macrophage spreading on antigen-antibody coated glass surfaces (351), but it differs from cobalt in being able to stimulate phagocytic activity and the oxygen uptake of dog granulocytes in vitro in the presence of 50-μM concentrations.

K. Chromium

Chromium is toxic to cultured alveolar macrophages (348) and to lymphocyte cultures at 10^{-4} M concentrations and it inhibits RNA synthesis and the proliferative stimulation of lymphocytes by LPS at concentrations as low as 10^{-7} M (343).

L. Mercury

Mercury, given in toxic dietary loads of 500 ppm diminished the resistance of chickens to *S. gallinarum* (336). In other studies, resistance of mice to enchephalomyocarditis virus was impaired (334, 368).

When mercury was added to the drinking water of rats for a 70-day period, achieving a total accumulated dose of 0.17 g, rabbits showed an inhibited serological response to a 3-wk series of formalinized pseudorabies virus inoculations, manifested by a diminished production of neutralizing antibodies in comparison to the antibody response of nonintoxicated normal controls (337). Diminished antibody responses have also been reported using other antigens in mercury-intoxicated animals (368–371). However, antibodies against cellular nucleochromatin may form in lead-intoxicated animals or humans which can lead, in turn, to immune complex-induced glomerulopathy (372).

Alterations in lymphoid architecture develop in mercury-intoxicated mice (371) and their splenic lymphocytes respond poorly to mitogens (341). Mercury also causes direct in vitro cytotoxicity (341, 348).

M. Vanadium

Vanadium toxicity caused by feeding 25-ppm dietary loads, diminished the resistance of chicks to *S. gallinarum* (336). Vanadium is also toxic in vitro to cells such as alveolar macrophages (348).

N. Gold

Gold compounds in concentrations of 5-200 μM in vitro can suppress the aggregation and degranulation of human neutrophils (373).

O. Iodine

Haggard et al. (374) administered oral iodine in different doses to calves during a 6-month period. The group receiving the highest dosage, 1.2 mg/day, exhibited a lowered antibody response to Brucella and Leptospira vaccines, diminished in vitro lymphocyte response to stimulation with PHA, a decreased DDH reaction to PHA, and diminished phagocytic activity of peripheral blood neutrophils. Dose-dependent immunosuppressive responses were also seen in other groups.

Iodine may also function in microbicidal activities. The neutrophilic peroxidase, myeloperoxidase, has potent in vitro antimicrobial activity against bacteria, fungi, viruses, and mycoplasma when combined with H_2O_2 and an oxidizable halide cofactor, such as iodide, bromide, chloride, or thiocyanate ions. Of these, the conversion of iodide to iodine is the most potent in terms of its bactericidal capacity. Such a system might be operative in vivo in activated neutrophils (375) with iodide supplied by thyroid hormones. Resting human neutrophils degrade T_4 and T_3 (376); this activity if markedly accelerated when the cells are activated by phagocytosis. Deiodination of T_4 is markedly enhanced in peripheral leukocytes of septic monkeys (377). Both T_3 and rT_3 can be detected as minor products of T_4 degradation and most of the liberated iodide from T_4 and T_3 can be recovered in monoiodotyrosine or as iodine; the latter is fixed in cytoplasmic sites which contain ingested bacteria (375). Further, leukocytes take up additional T_4 from the surrounding media when stimulated by phagocytosis (378). The T_4-deiodinating activity in human neutrophils resides in the granule fraction; characteristics of the deiodinative system suggest that it is enzymic in nature with a Km of approximately 10^{-6} M (379). Neither the superoxide radical nor H_2O_2 plays a major role in stimulating the deiodination of thyroid hormones, but once deiodination occurs, H_2O_2 appears to be more

important than the superoxide radical in subsequent iodination reactions within the cell (380). An accelerated deiodination of thyroid hormones also occurs during phagocytosis by monkey neutrophils and guinea pigs peritoneal macrophages (378, 381). In contrast, leukocytes obtained from patients with congenital chronic granulomatous disease are deficient in myeloperoxidase activity and H_2O_2 formation. The defective cells degrade thyroid hormones poorly during and after phagocytic stimulation (375).

P. Nickel

If added to the media of cultured cells, nickel can suppress the synthesis of interferon as induced by Newcastle disease virus (10).

Q. Silica

Microparticulate silica, if injected parenterally or inhaled as an aerosol, is rapidly taken up by phagocytic cells. Its toxicity for these cells appears to be selective and they are rapidly destroyed. No data were uncovered concerning an immunological effect of silicon preparations or dietary constituents.

R. Comments on trace elements

Despite recent research, available data about immune systeem interactions with iron and zinc are far from complete. The remainder of the field of trace element interactions is virtually unexplored. Basic reasons for the essentiality of some of these elements may well be defined by using immunological technologies to probe their molecular actions.

It will also be important to learn how to diagnose potential deficiencies of these hard to measure trace elements, especially since their depletion from body tissues may constitute an unrecognized coexisting problem during states of generalized PEM. Possibilities for trace metal excesses must also be considered, such as those which could occur through self-administration, through unrecognized contamination of processed food and beverages, or as a result of a prolonged accumulation during chronic hemodialysis (382, 383).

VII. Discussion

Additional work will be needed to clarify many of the unsettled points raised by this review. On the one hand, the complexities and subtle balances that exist within the immune system are only now becoming evident. On the other hand, many new leads have emerged to permit the further study of single nutrient actions at the molecular level. These sets of emerging research opportunities can now be brought together. Knowledge about lymphocyte biology is growing rapidly. In comparison to the brief survival time of most circulating white blood cells, lymphocytes are extremely long-lived. Nevertheless, their thin outer layer of carbohydrate (glycocalyx) is a highly dynamic structure with turnover times of 14 to 24 h; surface components lost by shedding into the surrounding microenvironment must be resynthesized continually (384). B-lymphocytes display about 10^5 molecules of immunoglobulin on their surface membranes, all of which exhibit the same limited antigenic specificity as the immunoglobulins secreted by the cell after stimulation. These surface proteins are also lost by shedding or enzymatic degradation and can be restored completely within 6 to 8 h (384). These factors, together with their ready responsiveness to blastogenic stimulation, create the present image of the lymphocyte as a highly active metabolic unit. Lymphocytes must therefore be regarded as "conspicuous consumers" in terms of the many individual nutrients they may require in order to function optimally.

No single micronutrient has been studied for all the immunological effects that can be measured. No study has attempted to determine if recognized genetic histocompatibility factors influence nutrient effects on immune function. The potentially different requirements of individual lymphocyte groups and subsets and of the phagocytic cell populations must be determined with respect to individual nutrients. The influence of single nutrients on the production of individual serum and secretory immunoglobulins has not yet been studied. The influences of individual nutrients on tumor immunology is only now beginning to be explored.

To be of optimal importance and fully interpretable, new studies must be well designed, and must include appropriate controls. Inanition controls are long recognized necessities whenever studies involve a single nutrient as the manipulated variable. This

requirement has most often been met by utilizing pair-fed controls. Even this approach is not without criticism, however, for the secondary metabolic consequences of a single nutrient deficit may not be matched adequately in a pair-fed population. Because infections cause important immune system responses, the occurrence of overt or subclinical infections must be excluded in all experimental groups included in a nutritional study, if the findings are to be ascribed to the manipulated nutrient. Other unrecognized but potentially important variables include both manipulative and environmental stresses as well as the influences of circadian rhythmicity.

Advances in medical and surgical technologies and in the nutritional sciences are making it possible for patients to survive in unique circumstances. Single nutrient excesses or deficits may develop during chronic hemodialysis (382, 383). The prolonged use of total iv alimentation can ultimately lead to unanticipated problems of single nutrient deficiencies such as those already experienced with zinc and chromium, or even to yet unrecognized single nutrient excesses. Attempts to raise infants with congenital inborn combined immunodeficiency disease in a totally isolated environment calls for the use of sterilized foods and provides another potential area for trace nutrient imbalances to occur. On the other hand, as recently learned in infants with congenital defects in neutrophil locomotion, single nutrient therapy may provide unique immunological benefits (91, 93). In addition, some food faddists are performing self-experimentation studies with combinations and doses of single nutrients never previously envisioned, and these too may result in functional immunological problems.

Of greater total importance than these relatively exotic possibilities are the unsolved immunological questions which arise during generalized nutritional deficiencies associated with famine and pestilence, such as those which currently affect millions of people. Deficiencies of iron, vitamin A, and possibly zinc often coexist with those of PEM. Each of these potential micronutrient deficiency states can contribute to the functional immunoincompetence of the starving patient. There is as yet no scientifically based rationale for initiating a program of nutritional rehabilitation to minimize the chances for creating immune system imbalances during refeeding, which could do further harm to the patient.

The nutritional role of cholesterol and the saturated and unsaturated fats pose continuing controversial problems of major importance. The potential immunological impact of these problems may be magnified clinically by the growing use of intravenous lipids as a source of metabolizable energy (385, 386). New questions have also emerged about minimal and optimal doses and combinations of vitamins and trace elements, and even about whether currents concepts of normal nutrition are truly optimal for immune system maturation and its eventual senescence. The role of single nutrients in each of these areas will require future attention.

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